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EFFECT OF THE DELIVERY ROUTE AND DOSE OF MULTIPOTENT MESENCHYMAL STEM CELLS ON THE EFFICACY OF CELL THERAPY (REVIEW)

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Multipotent mesenchymal stem cells (MMSCs) are known to be excellent therapeutic agents. Apart from their ability to differentiate into various cell types, and thus participate in the repair of injured tissues and organs, they can influence the regeneration process through secretion of paracrine factors. Thus, MMSC therapy represents a special type of medical intervention that has both a systemic range of therapeutic efficacy and local activity on individual sites of an organ. Over the past decades, MMSC therapy has continuously been in a cautious transition from research development to clinically approved therapies. Clinical trial data has shown that this therapy is rarely associated with severe adverse events, is well tolerated and quite safe in the short-term period. However, it has a number of limitations for use, mainly due to the risk of malignant transformation. The success of stem cell transplantation in the treatment of various diseases has been confirmed both in preclinical studies and in clinical practice. The main issues that arise when assessing the therapeutic efficacy of MMSC-associated therapy are the type of cells (adipogenic, bone marrow, etc.), delivery route, number of cells injected, and the optimal number of injections. There is a growing body of experimental and clinical evidence suggesting that both an adequate delivery route and an adequate dose can increase the likelihood of success of MMSC-associated. Each cell delivery route has costs and benefits. However, there is generally contradictory evidence on the comparative efficacy of different cell delivery routes. The optimal dose of transplanted cells is also debated, as high MMSC doses may increase the risks of complications and may not have the proper effect both when administered systemically and locally. These aspects require further systematization of available data to maximize the effect of cell therapy by selecting the safest and most appropriate approaches.

Keywords: multipotent mesenchymal stem cells, cell therapy, transplantation, delivery route, delivery dose.

INTRODUCTION

Modern breakthroughs in biotechnology, molecular and cellular biology have made stem cells one of the means for treating numerous diseases. Multipotent mesenchymal stem cells (MMSCs) have three main therapeutic effects: they differentiate and replace damaged tissue cells, they produce bioactive molecules, and they engage in intercellular communication and interact with immune cells [1–3].

MMSC-based therapy is generally considered a safe procedure with some limitations. The main risks associated with MMSC use are their possible oncogenicity (transformation into tumors, stimulation of tumor growth) [4, 5] and induction of severe pro-inflammatory processes [4] and fibrosis (transformation into myofibroblasts) [4, 6, 7]. Many studies have proposed algorithms to improve engraftment and differentiation of transplanted cells. While some strategies focus on increasing cell resistance to the microenvironment in recipient tissues, others try to increase cell survival after transplantation. These strategies can range from simple modification of culture conditions, known as cell preconditioning, to genetic modification of cells to avoid cellular senescence [8]. An essential element in improving the efficacy of cell therapy and reducing the risk of adverse events is the search for optimal delivery routes and doses for MMSCs. Despite many preclinical and clinical studies, the safety and efficacy of MMSC-related therapies remain problematic for clinical application [9, 10].

The **aim** of this work is to conduct an informational and analytical study of experimental and clinical data on the efficacy of various stem cell delivery routes and doses as a means of therapy for various diseases.

RESULTS AND DISCUSSION

MMSCs are typically administered intravenously or intra-arterially to achieve systemic effects on the body. When administered systemically, they can migrate directly (homing) to the site of injury in response to chemokine and cytokine secretions [11]. Although the exact mechanism of how MMSC home to sites of injury has not been fully understood, it is known that it is a multistep process in which chemotactic factors play a significant role [8]. Chemoattraction of MMSCs to the target tissue seems

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to be mainly mediated by stromal factor SDF-1/CXCR4 axis, but monocyte chemoattractant protein/CCR2 and hepatocyte protein as well as cytokines such as TGF-β1, IL-1β, TNF-α or G-CSF may also be involved in MMSC migration [12–14]. It is obvious that intravascular administration is the least invasive delivery route and, therefore, the most preferable from a clinical standpoint. However, this route has significant drawbacks. The major one is that a large number of injected cells can linger in the capillary network of lungs (first passage effect) and other organs, such as liver and kidneys. Considering that during transmigration, MMSCs should traverse membranes between vascular endothelial cells and the target tissue, it is obvious that delivering cells to target tissues through systemic infusion in the most efficient manner is rather difficult. At the same time, MMSCs can form microemboli, which create major consequences for organ function, given that the estimated cell diameter is 20–30 μm [8, 15]. There are serious concerns about the safety of this delivery method because studies on mice models have shown that in the intra-arterial delivery route, micro-occlusions are created, whose number was directly correlated with cell count [16]. Following intravenous injection, the presence of MMSCs or their detritus in the pulmonary capillary network is not just a temporary delay – macrophage phagocytosis occurs there, and the MMSC detritus is then further carried with blood to other organs [17]. One way to improve cell diffusion homing is the preconditioning of target tissues. For example, several studies have shown that administration of a number of hormones, chemokines, growth factors, and enzymes in experimental animals, as well as exposure to physical factors (ultrasound, irradiation), enhances MMSC migration to the injury site [8].

It should be noted that the injection procedure may be complicated by resuspension of cells in solutions that have low osmotic pressure, since mechanical stress can destroy cell membranes and lead to a significant proportion of the cell population dying [18].

It is established that the half-life of MMSCs after systemic administration is about 12 hours. However, as noted earlier, most of the cells are retained in the lungs, where they are utilized or, from where they migrate within 24 hours [2, 6, 8, 19]. After cells are injected directly into tissues against the background of venous blockade, detritus enters the bloodstream much later in smaller amounts. The main part of implanted MMSCs is found in other organs and tissues (liver, spleen, etc.) within 7 days [2, 20]. There is evidence that MMSCs can survive for 30 days after subcutaneous implantation [2, 21], but after that, they are undetectable in the liver, kidney and spleen [17]. After intravenous injection, cells can be detected in the lungs for 150 days [20].

Administration of MMSCs by surgical implantation or transendocardial injection has resulted in the retention of only 16% and 11% of MMSCs in the myocardium, respectively [5]. Intracoronary infusion also caused retention of 11% of MMSCs. Overall, approximately 0.1–2.7% of injected stem cells actually reach the target tissues [22]. Other implanted MMSCs mainly exert remote effects on regenerative processes via cytokines, exosomes and microvesicles, and exhibit mainly anti-inflammatory, immunomodulatory and anti-apoptotic effects [23]. It should be mentioned that less than 1% of cells are detected in the target organ in both animal research and some clinical trials using MMSCs for the therapy of osteogenesis imperfecta [15].

There are reports indicating that MMSCs, when delivered locally, mobilize progenitor cells to the injury site, thereby enhancing regenerative activity [5, 20, 21]. In doing so, they improve wound healing and skin graft survival. However, experimental studies have shown that MMSCs do not stay long in the injection site – most of them migrate into the surrounding tissues within 1 hour and are no longer detected in the injection site after 2 days [2, 21].

In a comparative study of three different MMSC delivery routes (intraperitoneal, intravenous and anal) in a mouse model of colitis, intraperitoneal delivery was shown to provide a higher MMSC content in organs and faster recovery of experimental animals [15]. The efficacy of therapy was evaluated by histological index, total body weight of animals and their survival rate.

Distribution and engraftment of MMSCs in organs were analyzed and quantified through isolation of the cells from green fluorescent protein (GFP+), as well as using near-infrared fluorescence imaging. There is evidence that intraperitoneally injected MMSCs aggregate with macrophages and lymphocytes in the abdominal cavity and secrete TSG-6 (tumor necrosis factor-inducible gene 6 protein), which is likely the main anti-inflammatory mechanism of MMSCs. Increased serum TSG-6 level was detected after MMSC transplantation, with the highest levels detected after intraperitoneal delivery.

It should be noted that the abdominal cavity contains many immune cells that can become components of MMSC aggregates. Such close intercellular cross-linking between MMSCs and immune cells may be another factor contributing to improved therapeutic effects [15]. It has also been observed that intraperitoneal injection provided better mucosal repair and higher cell engraftment in inflamed colon.

Intraperitoneal injection of MMSCs had a positive effect on recovery of mice with experimental spinal cord compression injury (SCI) [24]. The evaluation criterion was the effect of MMSC transplantation on white matter preservation. It was shown that experimental groups of animals that received MMSCs at a dose of 8×10^5 cells/mouse had a greater number of preserved fibers. In addition, these groups were characterized by higher levels of trophic factors (brain-derived neurotrophic factor, nerve growth factor, neurotrophin-3 and neurotrophin-4) in the

spinal cord, which improved motor activity. So, intraperitoneal or intravenous injections of MMSCs promoted favorable outcomes as a treatment for SCI without a significant statistical difference between the two. This supports the idea that these cells do not replace damaged spinal cord cells but act through local paracrine effects.

F. Yousefi et al. (2013) showed that intraperitoneal injection of MMSCs can reduce the number of inflammatory aggressor cells in the brain and improve clinical parameters in mice with experimental autoimmune encephalomyelitis [25].

There is evidence that intraperitoneal injection of MMSCs can suppress peritoneal inflammation by restoring the mesothelial layer and reducing complement activation in fungal or yeast peritonitis in rats; it almost completely prevents experimental autoimmune uveitis in mice by suppressing Th1/Th7 immune responses, protecting the retina against immune-mediated injury [24].

Wang et al. (2016) showed that the best outcomes in the treatment of experimental colitis were achieved by intraperitoneal transplantation of MMSCs [15]. It was found that GFP+ MMSCs migrated into inflamed colon and even traveled through the entire intestinal wall, reaching the luminal side. This finding is consistent with the results that show MMSCs injected intraperitoneally migrate and take root in an inflammatory colon [26]. Although the precise processes underlying this phenomenon are still unknown, it can be hypothesized that cytokines are involved in the process. It is known that genetic modification of MMSCs to increase CXCR4 expression enhances cell migration into the intestine in radiation enteritis, and consequently, improves condition. Experimental studies by Yang et al. (2019) showed that a single intraperitoneal injection of MMSCs (2×10^6 cells/mouse) dramatically improved clinical parameters of body weight and colon length, as well as ulcer size and histologic parameters in mice with colitis compared to those without [27].

Nevertheless, in some cases, intravenous injection has been found to be more successful than intraperitoneal injection [28]. This discrepancy may be due to different types of adipose tissue- and bone marrow-derived MMSCs that differ in proliferation rate, differentiation ability, cytokine secretome and chemokine receptor expression, which may affect migration, engraftment and even local function [29, 30]. There is evidence that bone marrow MMSCs-based therapies demonstrate the highest osteogenic potential in bone regeneration compared to MMSCs derived from other tissues [8]. Furthermore, the therapeutic effects of various MMSC sources and delivery methods on lung and cardiovascular injuries vary [31]. Therefore, while evaluating study findings and making specific therapeutic application choices, it is important to consider the biological differences between MMSCs and other sources. Meanwhile, there is a report that claims that because the immunophenotypes of bone marrow and adipose tissue stem cells are more than 90% identical, it is impossible to determine which stem cell source – adipose tissue or bone marrow – will yield the best outcomes for cell therapy [9]. Besides, when assessing efficacy, it is important to consider that MMSCs may exert their therapeutic effects distally through modulatory cytokines [32].

Intramuscular injection of MMSCs has been proposed as a better alternative to intravenous administration [33]. Braid et al. (2018) report that while cells injected intravenously were undetectable as early as a few days after injection, and cells delivered intraperitoneally and subcutaneously were detectable within 3–4 weeks, MMSCs injected intramuscularly survived *in situ* for more than 5 months. Allogeneic single intramuscular transplantation of umbilical cord-derived MMSCs to rats with simulated hind limb ischemia promoted functional and morphological recovery of ischemic skeletal muscle tissue [34]. At the same time, the cells injected into experimental animals stimulated angiogenesis in the injury site.

In general, quite numerous experimental studies have shown MMSCs to improve functional recovery in ischemic stroke; this is attributed to the ability of MMSCs to enhance the endogenous regenerative potential of nervous tissue [35, 36]. This is due to the action of bioactive substances released by the cells, which activate and stimulate other cell types [37]. The choice of an optimal delivery route for MMSCs in cerebral ischemia depends on the type of central nervous system (CNS) injury (focal or multifocal). The peculiarities of focal CNS injury suggest that the most appropriate way may be intracerebral cell transplantation directly into the injury site, and in case of multiple lesion areas – systemic intravascular or endolumbar injection [37].

It has been shown that intraportal and intravenous administration of MMSCs in experimental liver cirrhosis promotes faster recovery of liver function. Moreover, liver weight decreased the most with intraportal injection of stem cells [38]. Administration of acridine orange-labeled MMSCs intravenously, intraperitoneally, into the hepatic artery or portal vein at a dose of 4×10^6 cells/kg body weight showed a significant increase in cell count in the liver after its subtotal resection regardless of the injection method [39]. At the same time, intraperitoneal delivery was characterized as the least effective.

When correcting diabetes in an experiment, intravenous injection of bone marrow-derived MMSCs statistically significantly reduced glucose levels in mice of the experimental group compared to the control group [40].

It was found that MMSC implantation promotes neurological recovery in a rat model of traumatic brain injury (TBI) [41]. Intravenous injection of cells into rats reduced the number of microglia and other inflammatory cells and production of proinflammatory cytokines. It also stimulated the synthesis of anti-inflammatory

cytokines leading to inhibition of inflammatory reactions caused by TBI [42].

In clinical practice, the multipotent and secretory potential of MMSCs finds application in the field of regenerative medicine for restoration of tissue structures of the body that were damaged by injuries or that developed pathology (combustiology, traumatology, dentistry, etc.). Here, introduction of stem cells in the patient is usually done intravenously to provide a systemic effect on the patient's body [2, 23]. Direct comparison of delivery methods is often absent here due to logistical problems [5]. An adequate dose of MMSCs administered once has been shown to have a positive clinical effect in 3–6 months (and beyond). In certain cases, however, a repeat course of treatment in 1–2 weeks (4–6 months) is necessary to reach the desired therapeutic outcome [2, 43–46].

The experience of intravenous injection of MMSCs in patients with chronic heart failure (CHF) against the background of ischemic heart disease is presented. It was shown that intravenous administration of autologous MMSCs at a dose of 50×10^6 cells in combination with standard drug therapy improves basic hemodynamic parameters and reduces the level of biochemical markers of CHF [47]. In addition, there is evidence showing the successful application of intracoronary and intramyocardial delivery routes in the treatment of ischemic conditions in clinical practice [48].

To date, 125 clinical trials have been conducted using MMSCs in neurologic diseases [31], including the treatment of TBI. Injection of autologous bone marrow-derived MMSCs in patients in the subacute phase of TBI improved neurological function in 40% of patients [49, 50], promoted recovery of consciousness, motor and cognitive functions [51]. Intravenous delivery is used for TBI therapy, since MMSC delivery via the intracerebral route is considered to be the most effective, but also the most invasive [31].

The outcomes of clinical trials on cirrhosis therapy using MMSCs are contradictory and occasionally inconsistent with the results of experimental studies [52]. Nevertheless, uncontrolled clinical studies have demonstrated that introduction of autologous MMSCs into the hepatic arterial bed through endovascular surgery is safe, well tolerated, and provides a positive effect in patients with cirrhosis of various etiologies [53].

For the treatment of patients with knee joint conditions, delivery of autologous MMSCs was via intraarticular injection of cell culture isolated from different sources [9, 54]. It was noted that long-term follow-up parameters were significantly superior to those in the control group receiving conventional treatment [54].

The use of intraperitoneal cell transplantation in clinical practice has significant limitations due to possible complications. These complications include catheter infection and mechanical damage to intraperitoneal structures [15]. At the same time, it should be noted that high peritoneal vascularization allows a greater number of transplanted cells to simultaneously gain access to the lymphatic and circulatory systems, which certainly promotes engraftment in the injury and inflammation sites [24]. It is obvious that the trend to expand the use of intraperitoneal injections in cell therapy applications will increase and will influence the intensification of innovative developments aimed at preventing complications.

Another crucial factor influencing the therapeutic efficacy of MMSCs is the number of cells injected. The quantity of cells that reach the damage site increases with an increase in the initial dose of cells delivered. Various MMSC dosages have been found to be effective in experimental investigations. Doses from 3×10^5 cells/mouse to 2×10^6 cells/mouse appear in the protocols of experimental studies [15, 24, 25]. Sometimes, to observe any effect, larger doses, up to 5×10^6 cells/mouse, are used [37, 55]. However, the researchers conclude that intravenous injection of MMSCs at a high dose (up to 1×10^7 cells/mouse) can increase mortality in mice due to potential pulmonary embolism.

Usually, MMSC doses in the range of 5×10^5 to 5×10^6 cells/mouse are used to achieve therapeutic effect in experimental rats [55, 56]. At the same time, cell survival after transplantation into recipient tissue depends not only on dose, but also on duration and conditions of cultivation, such as presence of serum or oxygen, mechanical stress during implantation or cell death due to lack of fixation [56, 57]. There is an opinion that regional injection (endolumbar, intraperitoneal, intramuscular) results in a tenfold decrease in the therapeutic dose of cells [2].

A significant challenge lies with translating the experimental dosage of the cell product for use in clinical practice. As mentioned above, the commonly used cell dose is 1×10^6 cells/mouse (body weight 30 g), which is equivalent to 33×10^6 cells/kg or approximately 2.3 billion cells for a 70 kg adult [14]. Researchers have suggested that high cell doses may increase the risks of complications, including alloimmunization when using allogeneic MMSCs, and may not have the proper effect when injected both intravenously and topically [2, 22, 58]. Nonetheless, the concept of "optimal dose" of systemically injected MMSCs in clinical practice does not exist yet since there is no clear dose-effect correlation [8]. Today, the standard dose is 1–2 million cells per kg of weight [2, 58]. Moreover, compared to standard doses, 5–10 higher doses of cells are used during the cell therapy process for newborns – but usually just once [2, 43].

Using autologous MMSCs, experimental and clinical simulations were used to examine the efficacy of various cell delivery methods in the treatment of Parkinson's disease [59]. When compared to baseline data, an intravenous injection of 160,000 cells/kg weight (low dose) resulted in a statistically significant decrease in motor disorders. At the same time, transnasal injection

in a similar dose in patients of the other group had a similar effect. These findings suggested that while designing long-term maintenance therapy for Parkinson's disease, the efficacy of minimally invasive techniques for delivering low-dose MMSCs should be taken into account.

CONCLUSION

Most preclinical and clinical studies have shown that MMSC implantation is effective, safe and well tolerated. Analysis of data from scholarly publications indicates that there is ongoing research being done to find the best cell delivery dosages and routes. It should be noted that attempts are now being undertaken to develop the option of using exosomes and extracellular vesicles as a cell-free means to realize the features of MMSCs, with the goal of removing any potential side effects. It is obvious that application of a cellular product in practical healthcare requires maximum adaptation to the type of disease or injury in terms of the choice of implantable MMSC doses and their delivery routes given the need to ground the therapeutic strategy with a clear and thorough understanding of the disease mechanisms. In general, research findings and the opinions of different authors on this issue are far from being ambiguous and sometimes contradictory. As such, a versatile study of the therapeutic potential of MMSCs remains pertinent.

The authors declare no conflict of interest.

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