Potential clinical applications of bone marrow-derived mesenchymal stem cells in bone regeneration

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Summary

Bone marrow (BM) contains a small resident cell population referred to as ‘multipotent mesenchymal stem cells’ (MSC). These adherent cells could be isolated and expanded in simple culture media and may differentiate in adipogenic or osteogenic pathway. So far an opportunity of MSC differentiation to hepatocytes, brain, or renal cells is not proven yet. Meanwhile, two potential clinical applications are considered for MSC: (1) as a tool for immune modulation in graft-versus-host disease (GVHD) and autoimmune diseases, or, (2) as a potential source of growth-promoting factors in specialized tissues. This heterogenous population may support hematopoiesis by secreting growth factors, cytokines and other biologically active substances. Upon injection, MSCs are able to migrate into damaged tissues, thus promoting their repair. However, only small MSC fraction may reach bone marrow niches following intravenous infusion. Multiple experiments with MSCs in different injury models show their ability to suppress apoptosis initiated by hypoxia, chemical agents/acidity and other deteriorating factors. This protective effect is mediated by a number of secreted growth factors, e.g., granulocyte-macrophage colony-stimulating growth factor (GM-CSF). A big number of clinical trials show high level of safety for the MSC therapy. Both clinical and experimental studies demonstrated only weak immunogenic effects of allogeneic MSC upon injection into immunocompetent recipients. At the present time, injections of in vitro expanded MSCs were performed in the patients developing acute GVHD after hematopoietic stem cell transplantation (HSCT), and in some autoimmune disorders.

Over last decade, several studies concerned potentially curative effects of MSCs injected into affected bone areas in the patients with osteogenesis imperfecta (OI), a severe inherited disease with altered collagen structure resulting into increased bone fragility. Here we present a synopsis of clinical protocol aimed for assessing safety, immunogenicity, and clinical effects of MSC injected to the OI patients during corrective osteotomy. One may suggest that a minor MSC subpopulation may migrate to the damaged areas differentiating to chondrocytes and osteoblasts, and, hence, contributing to the bone repair.

Keywords

Mesenchymal stem cells, biodistribution, secreted factors, ex vivo expansion, clinical applications, osteogenesis imperfecta.

Introduction

In vitro expanded mesenchymal stem cells (MSCs) are currently tested as a promising tool for, e.g., prophylaxis of acute graft-versus-host disease (aGvHD) and organ rejection. More recent studies are performed, concerning feasibility of MSC-based therapies in heart insufficiency and acute renal failure [108]. Despite multiple studies, the main problem is to choose optimal and standard growth supplements in order to obtain reproducible results of the differentiation experiments. Meanwhile, much more expectations are connected with their abilities of in vitro differentiation into various specialized cell types. Two main pathways are easily detected under conventional culture conditions, i.e., adipogenic and osteogenic differentiation modes. Moreover, some specific growth factors may drive MSC to differentiate into the cells of other lineages, e.g., hepatocytes, brain, or renal...
cells. There are, however, some doubts on reproducibility of such trans-differentiation events for a sufficient fraction of proliferating MSCs.

Most clinical trials with MSC injections are aimed for treatment of autoimmune and chronic inflammatory disorders [19]. Moreover, these cell populations are regarded as a potential source for regeneration of hematopoietic and other tissues, due to a number of biologically active factors produced by MSCs, as shown by in vitro and in vivo studies [55].

**Bone marrow-derived MSCs**

Bone marrow (BM) represents a reservoir of different-type stem cells and precursor cells. Along with hematopoietic stem cells, the marrow contains a cell population which was previously referred to as ‘mesenchymal stromal cells’, ‘bone marrow stromal cells’, or ‘marrow multipotent mesenchymal stem cells’ [31]. All these denominations are covered with an acronym ‘BM MSC’. These cells comprise a small fraction (0.001 to 0.01%) of the entire BM nucleated population, but they could be isolated and subject to expansion on the basis of their ability to adhere to different surfaces [18]. BM MSC are multipotent and are able to differentiate into precursors of osteoblasts, adipocytes and chondrocytes [74]. This heterogenous cell population takes active part in hematopoietic regulation, by secreting growth factors, cytokines and other biologically active substances, as well as by intercellular and cell/matrix interactions. BM MSCs exhibit spindle-like morphology, CD73, CD90 and CD105 expression, along with negativity for hematopoietic cell markers (CD45, CD34, CD14 etc.). Numerous studies have shown that the ex vivo expanded MSCs, by systemic or local injection, are able to migrate into damaged tissues and organs and actively participate in tissue repair processes [11,77,93,98,107]. Moreover, BM MSC possess low immunogenic potential and suppress immune response, both in vitro and in vivo [2,45,47,94].

Historically, MSCs were for the first time isolated from bone marrow. Nevertheless, in further studies, MSC with similar characteristics were obtained from other organs and tissues, including subcutaneous fat, umbilical blood, placenta etc. [39,57]. Morphology, phenotype and functional properties of MSC from other sources are largely similar to BM MSC.

MSC abilities for a multi-lineage differentiation are actively studied since their discovery by A.Ya.Friedenstein in 60’s of XX century [1]. Numerous studies have demonstrated that the ex vivo cultured MSCs are able for in vitro and in vivo differentiation to the terminally differentiated cells of mesenchymal lineage, e.g. osteoblasts/osteocytes, chondrocytes, adipocytes, myocytes and stromal cells that may support hematopoiesis [9,10,14,20,21,37,76].

There are some problems with in vivo fate of the in vitro expanded MSCs isolated for subsequent therapeutic use. In fact, sufficient complement-mediated MSC cytotoxicity and lysis were revealed following their short-term in vitro treatment with fresh isologous serum [63]. This cytotoxic effect was abolished by the serum pretreatment with anti-C5 monoclonal antibody (Eculizumab), or divalent ion deprivation. Therefore, one should expect sufficient loss of survival for intravenously infused MSCs, as it was shown in previous studies, thus sufficiently changing their homing pattern and biological activity. Moreover, a big part of infused MSCs is entrapped in small vessels of lungs and, therefore, does not reach hematopoietic tissues or other target regions [43]. As a result, only small MSC fraction may reach bone marrow niches while homing in lungs spleen etc. Hence, MSC persistence in the body is rather short-timed, with only small amounts residing for months.

**Animal models**

Most studies concerning distribution of ex vivo cultured and post-labeled human MSCs after their intravenous administration to animals (i.e., mice with immune deficiency) have shown that a vast majority of the cells was captured and entrapped in lungs within 15 to 30 min. [48]. Meanwhile, halflife time of the cells in lungs was about 24 hours. Histological examination of the lung samples demonstrated MSC-associated embolism in small arteries, along with progressing apoptosis of the most MSCs. [49]. Only a small fraction of injected cells entered blood circulation, being distributed into different organs, e.g., liver, lungs, kidneys, bone marrow etc. At 48 and 96 hours post-injection, respectively, 0.04% and 0.01% of initially applied cells were detectable in these organs and tissues. Thereby, the MSC distribution patterns did not differ from those obtained upon injection of human mammary carcinoma cells [48].

The data concerning long-term MSC persistence showed sufficient inter-study differences, probably, due to the label type chosen. In some studies, human cells were revealed only in spleen by the day 7, but not after 3 months [44]. Meanwhile, other detection techniques have shown that small MSC amounts may persist in bones, cartilages, bone marrow, muscles and spleen for several months [3].

**Human studies**

By now, only few studies were dedicated to distribution of MSC following intravenous injection to humans. Appropriate results confirm a similar distribution pattern, i.e., cell entrapment in pulmonary microvascular network early after infusion, and probable differences in their subsequent recirculation, which may be connected with species-specific anatomical and physiological features, disease states in the persons under study, or alternative techniques of cell detection. E.g., radioindium-labeled MSCs were infused i.v. to the patients with liver cirrhosis [22]. At early terms, the cells were accumulated in lungs, however, they were displaced to liver and spleen within hours and days. Radioactivity in lungs diminished, respectively, from 33.5% to 2% in lungs, while being increased in spleen from 2% to 42%.

Long-term persistence of allogeneic MSCs was studied in patients with acute graft-versus-host disease (GvHD), by means of donor DNA detection in biopsies form different tissues [100]. In 8 of 13 patients, minimal amounts of donor DNA were detected in one or more samples obtained,
mostly, from lungs, spleen, lymph nodes and small intestine, within 50 days after last infusion. Quantitative analysis has shown that the donor cell numbers in these tissues did not typically exceed 0.001%.

Safety of therapy with ex vivo cultured MSC

Ex vivo grown MSC from bone marrow and other sources have been actively tested in clinical trials, from the 1990s. At the present time, more than 400 clinical trials are registered in different databases [51]. Analysis of short- and long-term effects revealed a high-level safety of this therapeutic approach. A special meta-analysis concerned adverse effects after MSC injections performed in 1012 patients enrolled into 26 clinical studies [42]. Allogeneic HLA-compatible, or HLA-mismatched MSC were infused in 56% of the studies included. No correlations were revealed between MSC infusions and acute posttransfusion reactions, organ-specific complications, infections, development or progression of malignancies and/or lethal outcomes. A transitory fever was the only significant adverse effect that could be ascribed to MSC injections. It was documented for 30 to 40% of the cases in randomized studies, when applying both autologous and allogeneic cells.

Underlying mechanisms of MSC therapeutic effects

One may discern two main mechanisms underlying the therapeutic effects observed after MSC injection. The first mechanism implies MSC proliferation and differentiation into various cell types which replenish and/or replace functional cells lost due to certain pathological process or medical influence. The second mechanism presumes trophic and immunomodulatory effects exerted by MSC upon surrounding and remote cells and tissues, due to wide-spectrum secretion of biologically active substances as well as microvesicles and apoptosis products released into extracellular space intercellular exchange. Immediate therapeutic effects observed upon BM MSC injection are mostly mediated by soluble factors (cytokines, growth factors, low-molecular compounds) produced by the BM MSC or other cells upon their interactions with BM MSC [2; 64] and microvesicles, and due to close contacts, e.g., with hematopoietic cells [72, 12, 102].

MSCs secrete a number of soluble substances (cytokines, growth factors, low-molecular compounds) which exert direct or indirect influence upon surrounding and distant cells and tissues. The MSC-mediated endocrine and paracrine effects may be divided into trophic and immunomodulatory ones. [11]. In turn, the MSC trophic effects are under lied by their ability to prevent apoptosis of neighbor cells, induce proliferation and differentiation of endogenous precursor cells, as well as to initiate angiogenesis. At the present time, some distinct factors mediating MSC effects are partially identified (Table 1).

Immunomodulatory activity of MSC is exerted via inhibition of CD4+ and CD8+ T cell, and NK cell proliferation, decreased Ig production by plasmocytes, inhibition of dendritic cell proliferation, and stimulation of regulatory T cell proliferation. These effects are performed by a number of enzymes and secreted factors: prostaglandin E2 (PGE-2), soluble leukocyte antigen (HLA-G5), hepatocyte growth factor (HGF), inducible NO synthase (iNO), indole-2,3-deoxygenase (IDO), transforming growth factor beta (TGF-b), leukemia-inhibiting factor (LIF), and interleukin 10 (IL-10).

Multiple experiments concerning MSC effects in acute injury models of different organs, tissues and cells have shown that MSCs are able to prevent massive apoptotic cell death. MSC suppress apoptosis initiated by hypoxia, chemical factors/acidity, mechanical damage, and ionizing irradiation [62]. This protective effect is mediated by some key secreted molecules. e.g., vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF-I), stanniocalcin-1, transforming growth factor-beta (TGF-b), and granulocyte-macrophage colony-stimulating growth factor (GM-CSF).

Extracellular matrix molecules, (VEGF), (IGF-I), placental growth factor (PIGF), macrophage chemotaxis factor-1 (MCP-1), fibroblast growth factor (bFGF), and interleukin 6 (IL-6), secreted MSC are initiating angiogenesis. In turn, restoration of blood circulation in the injured tissues maya represent a fundamental factor for their successful reconstitution.

Endogenous precursor cells activated and attracted to injured sites from the surrounding tissues are playing a major role in repair processes. They migrate to the damaged areas and are the main source of the newly formed differentiated cells replacing the lost ones. Nevertheless, the efficiency of this process is often insufficient, especially in cases of severe injuries, when an external stimulation is necessary. MSC-secreted factors, e.g., stem cell factor (SCF), leukemia-inhibiting factor (LIF), M-CSF; stroma-derived factor (SDF-1) and angiopoetin-1 favor cell survival, proliferation and differentiation of tissue-specific endogenous precursor cells.

Table 1. Immunomodulatory factors released by in vitro cultured MSCs

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<thead>
<tr>
<th>Biological effects</th>
<th>Molecules produced [references]</th>
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<tr>
<td>Apoptosis prevention</td>
<td>VEGF [81,99], HGF [81,99], IGF-1 [99], Stanniocalcin-1 [8], TGF-b [81], bFGF [81], GM-CSF[81]</td>
</tr>
<tr>
<td>Immunomodulatory effect</td>
<td>PGE-2 [58,92], TGF-b [16,92], HGF [16], mPCL2 [79], IDO [60], iNOS [86], HLA-G5 [65], LIF [15,66]</td>
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Choice of MSC donor and immunogenicity of allogeneic MSC

MSCs may be harvested from the patient, then expanded and returned in autologous manner. However, MSCs for autotransplants should be sometimes isolated from senior people, diseased individuals, and females with suppressed osteogenic potential. Therefore, some benefits from usage of allogeneic MSCs are supposed in a review by Kovach et al. [41]. E.g., they suppose allogeneic MSCs isolated from young, healthy males to be optimal grafts for boosting bone repair in these populations at risk. However, one should account for non-predictable immune reactions, since some animal studies suggest that use of allogeneic MSCs is not feasible owing to immune response of the recipients to transplanted MSCs.

Ex vivo cultured MSC possess a pronounced immunomodulatory activity, express modest amounts of MHC I molecules, and do not express MHC II [5, 45]. These properties allow to suggest a low immunogenic potential of allogeneic MSC, as confirmed by some experimental studies, both in vitro and in vivo [4, 78, 85]. On other hand, proinflammatory cytokines induce higher MHC I levels, and MHC II antigen expression [71]. In rodent experiments, it was demonstrated that systemic infusion of allogeneic MSC is accompanied by alloimmune response [68, 109]. However, if compared with other cell types, the alloimmune response to MSC is more delayed and less pronounced, and allogeneic MSC may persist in the organism of immunocompetent recipient for a much longer time [109].

Some cautions concerning immunosuppressive effects of allogeneic MSC transplants arise from experimental study by Prigozhina et al. [75]. Immunosuppressive properties of MSCs in vivo were tested by a well-known model of ectopic bone formation in both syngeneic and allogeneic murine recipients. MSCs from different sources were implanted with neutral bone scaffold under the kidney capsule. Bone development was observed in only the syngeneic hosts, whereas the allogeneic hosts experienced transplant rejection. This data argue for perturbed in vivo immune interactions of MSCs in allogeneic recipients.

Meanwhile clinical studies in humans have shown that allogeneic MSC could be safely injected to immunocompetent recipient without development of clinically significant alloimmune reaction [6, 26, 27]. In spite of big number of trials, there are no convincing data which presume production of donor-specific antibodies (including HLA-specific) after systemic injections of allogeneic MSC. Moreover, no sufficient differences were detectable, when comparing therapeutic efficiency of auto- and allogeneic MSC [5, 26]. Hence, the issue of MSC immunogenicity and its influence upon therapeutic efficiency with allogeneic MSC remains unsolved and needs further studies.

Directed MSC migration

It was shown in several experimental animal studies that, at least, a part of MSCs arriving from lungs may migrate to the foci of injury/inflammation [7, 35, 36] and, hence, accumulate at these sites in greater concentrations than in intact tissues. A key role in the directed migration of MSC belongs to chemokines and adhesion molecules. Their induced expression is initiated in the cells involved into inflammation at the injured sites. At least, a fraction of MSCs (3-4%) is expressing the CXCR4 receptor which provides cellular chemotaxis along SDF-1 concentration gradient [106], thus playing a key role in migration of other cell types, e.g., hematopoietic and endothelial populations [44, 90]. Along with CXCR4, the MSC express receptors for other chemokines, i.e., CCR1, CCR4, CCR7, CCR10, CCR9, CXCR5 or CXCR6 [29, 101]. Directed MSC migration is performed due to the expression of some adhesion molecules on their surface, i.e., integrin beta1, and integrin alpha4. Inhibition of these molecules blocks the migration process [33, 84]. Moreover, MSC may express a number of matrix metalloproteinases, thus allowing the cells to migrate in extracellular matrix [83].

Time course of osteogenic effects in regenerating bones and MSC-derived factors

Bone injury and its repair is a multistep process which exhibits different patterns of bioactive molecules released at the damaged site, especially, in the cartilage growth plate which is the bone growth area in children. Depending on the terms post-fracture, osteogenesis strongly depends on specific growth factors released by cellular microenvironment [13]. The authors discern four stages of repair mechanisms, i.e., inflammatory, fibrogenic, osteogenic and remodeling phases. Inflammatory cytokines, e.g., TNF alpha, IL- beta exert strong regulating effects at the initial post-injury phase, whereas chemokines, PDGF and FGF2 are active at the fibrogenic stage. Specifically, transforming growth factor beta1 (TGFβ1) plays a critical role in bone reconstitution due to its potent chemotactic and proliferative effect on mesenchymal

<table>
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<tr>
<td>Antifibrotic effect</td>
<td>bFGF [95], HGF [95], Adrenomedullin [50]</td>
</tr>
<tr>
<td>Stimulation of proliferation and differentiation of endogenous precursor cells</td>
<td>SCF, LIF IL-6, M-CSF [28,56], SDF-1 [70,96], Angiopoietin-1 [70]</td>
</tr>
<tr>
<td>Angiogenesis stimulation</td>
<td>bFGF [40], VEGF [32,40], PI GF [40], MCP-1 [32,40], IL-6 [32], extracellular matrix components [91]</td>
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stem cells, therefore promoting accumulation of bone-forming tissues at the injured site. In addition, TGFβ1 induces production of ECM components, e.g., collagen, osteopontin, and alkaline phosphatase from MSCs and osteoblasts. Bone morphogenetic proteins (BMPs) also belong to the TGF-β superfamily and are additional growth factors for mesenchymal stem cells that induce both osteogenesis and angiogenesis. For more recent data see a review by Zigdon-Gilad et al. [110]. By these mechanisms, BMPs are involved at all phases of the cartilage and bone repair.

Likewise, several angiogenesis factors are important at the osteogenic response. MSCs may release angiogenic factors [angiopoietin (Ang)-1, -2, Anglike-1, -2, -3, -4, VEGF, and fibroblast growth factor-2] that attract resident MSCs and promote local angiogenesis, a condition sine qua non for renewal of osteoid structures [73].

### Potential MSC applications in osteogenesis imperfecta

Some studies concerned engraftment of ex vivo cultured syngeneic MSC in a murine model of osteogenesis imperfecta (OI), an inherited collagen synthesis disorder. Upon postnatal intravenous injection of the MSCs, only negligible numbers of osteoblasts (<2%) of donor origin were detected in recipient mice [17, 71], or their total absence [34]. Moreover, despite minimal amounts of donor osteoblasts (ca.1%) in homozygous oim/oim mice, no signs of a2 collagen type I chains were found in the bone tissue samples [71]. These data are indicative for a potentially low efficiency of MSC infusions, as a replacement cell therapy in (OI). Worth of note, despite lacking MSC engraftment, the oim/oim mice exhibited a notable increase in linear bone growth and total body mass as compared with mice from control group. The workers presumed this effect to be determined by chondrocyte proliferation in epiphyseal plates of the tubulous bones, due to some indirect paracrine effects of the MSCs injected.

Despite low efficiency of the MSC systemic infusions to intact bones, the MSC engraftment rates may be sufficiently higher if delivered to regenerating bone after its fracture. This depends on the well-known active migration of mesenchymal precursor cells from periost and other surrounding tissues, e.g., to blood circulation and repairing bone areas [87]. Several studies have shown that a small number of intravenously injected MSC could migrate to the fracture zones, later being differentiated to chondrocytes and osteoblasts which took active part in the tissue repair and bone callus formation [25, 69]. Thereby, the CXCR4-expressing MSC represented the only directly migrating population [25]. The MSC provided a significant improvement of the fracture healing, due to increased mass of regenerating chondrogenic and bone tissues, and development of harder bone calluses, when compared with control, non-MSC-treated, mice. Along with direct differentiation to chondroblastic and osteoblastic cells, the MSCs exerted a favorable effect upon repair, by means of local and systemic immunomodulatory and trophic effects promoting further survival and proliferation of endogenous mesenchymal precursor cells, faster transition from inflammatory phase and more rapid development of the callus and bone repair, due to in increased new bone formation tested by different visualization techniques (17.8%; 95% CI, 10.54; 25.03; P<0.001), and confirmed by available data on increased bone mineral density following MSC treatment. Some differences depended on cellularity of the transplant. Usage of matrix scaffolds seemed to be more effective than direct cell injection. Note worthy, the effects of MSC treatment proved to be diminished after 12 weeks post-transplant, thus presuming a need for repeated cell injections at longer terms.

### Pre-clinical studies of MSCs in bone regeneration models

A comprehensive systematic review of 20 selected preclinical studies involving large animals (dogs, sheeps, rabbits) with bone defects was published by a group from China [52]. The experimental procedures included, mainly, implants of scaffolds seeded by fat- or bone marrow-derived MSCs, or direct injections of MSC into the injury site. Observation terms varied between 12 and 36 weeks. Forest plot data analysis showed a significant beneficial effect of stem cell therapy in increasing new bone formation tested by different visualization techniques (17.8%; 95% CI, 10.54; 25.03; P<0.001), and confirmed by available data on increased bone mineral density following MSC treatment. Some differences depended on cellularity of the transplant. Usage of matrix scaffolds seemed to be more effective than direct cell injection. Note worthy, the effects of MSC treatment proved to be diminished after 12 weeks post-transplant, thus presuming a need for repeated cell injections at longer terms.

### Preliminary clinical data

At present time, only two study groups have published data on clinical application of systemic MSC infusions for treatment of OI patients. In the study by Horwitz et al. [30], six patients with type 3 OI, after previous allogeneic bone marrow transplantation, were subjected to double MSC in-
fusions from the same donors at a dose of 1-5×10⁶ cells/kg body weight. In five patients of six, an accelerated growth dynamics was observed within 4 to 6 weeks after infusions. The improvement comprised 60% to 94% (a mean of 70%) of the expected median values for healthy sex- and age-matched children. As compared with 0% to 40% (a mean of 20%), that were observed during 6 months preceding the infusions. Horwitz and colleagues have also launched a more extensive study with 15 patients, who received regular infusions of allogeneic or syngeneic MSC once every 4 months over a total of 20 months. However, the results of this study are not published so far.

Another group of investigators has published two cases of allogeneic MSC infusions to 2 patients with OI (types III and IV), who were diagnosed prenatally [24, 46]. This study had some specific features:

- Allogeneic MSC derived from fetal liver were used as a grafting material due to their higher potential for proliferation and multilineage differentiation, as compared with MSC from adults [23].
- The first infusion was performed at the intrauterine stage (into the umbilical vein), hence, the infused cells got directly to systemic circulation, avoiding the pulmonary circuit.
- At the moment of MSC infusions, both patients had multiple bone fractures, according to ultrasound data. Probably, these aspects of the study could predetermine high level of chimerism (up to 7-15%) in osteoblastic cellular lineage as seen from the bone sample testing. However, high chimerism levels proved to be transient and further dropped down to undetectable values. In both cases, the infusions were accompanied by total healing of the fractures and successful deliveries. Repeated infusions were performed post partum, at the age of 8 years and 19 months, primarily, due to stunting growth. MSC infusions were associated with resuming growth in both patients.

We have not find any works concerning systemic MSC infusions for immediate treatment of children with OI after corrective osteotomy of femoral and/or tibial bone.

Rationale and design of the ongoing study

Despite some favorable effects of the biphosphonate-type bone resorption inhibitors (e.g., increased bone mineral density and decreased fracture incidence), there is no effective treatment aimed for restoration of linear bone growth and prevention of the bone deformities in the patients with childhood OI. Moreover, the biphosphonate therapy is poorly compatible with corrective osteotomy, since these drugs suppress bone tissue remodeling, thus causing delayed healing of the osteotomy site [61]. Hence, a clear need for novel therapeutic approaches still exists for this group of patients.

On the basis of experimental and primary clinical data, we suggest that allogeneic MSC infusion should be a safe procedure, and, moreover, a stimulation of chondrocyte prolifera-

tion could be achieved in epiphyseal plates of the long bones, due to paracrine/endocrine effects of MSC infusions, thus leading to restoration of linear bone growth in pediatric patients with moderate-to-severe clinical forms of OI. Increase of the bone mass, improved quality of osteogenetic tissue and its higher mineral density could be achieved via systemic and local immunomodulatory and trophic effects of MSCs, their differentiation to chondroblastic and osteoblastic lineages, and probably, due to sufficient synthesis of normal type I collagen. These effects will cause increased strength of the bones subjected to surgical interventions. In clinical aspects, these events will lead to decreased incidence of bone fractures, accelerated consolidation and lower recurrence of bone deformities.

Allogeneic MSC are planned to be used in the present study. This is due to ability of such donor cells to produce normal type I collagen, if differentiating to osteoblastic lineage. On the other side, it is well known that occurrence of alloimmune response may be a limiting factor of cell therapy efficiency, since its long-range goal is to replace and restore cells and tissues by the donor cells. Therefore, an alloreactive immune response may prevent a long-term persistence of the donor osteoblasts and normal collagen synthesis. Nevertheless, MSCs are known to be only weakly immunogenic, and so far, according to numerous clinical trials with allogeneic cells, there are no sufficient clinical data indicative for a pronounced alloimmune response arising after systemic MSC injection (for details see under “Allogeneic MSC immunogenicity”). Moreover, some experimental results show that, in cases of alloimmune reactions towards MSC, such response is relatively delayed, and, hence, a period from cell injection to immune rejection signs is prolonged up to 20 days [109]. This time interval is sufficient for MSC migration to the osteotomy area and their differentiation to osteoblasts actively producing normal type I collagen. Besides that, we suppose that a therapeutic action of injected MSCs is determined, mainly, by systemic and local trophic/immunomodulatory effects that are produced by the donor cells within short terms post-infusion. Hence, the time intervals necessary for the basic therapeutic actions of the MSCs (hours to days post-injection), are not interfering with their probable rejection terms, due to allo-immune mechanisms (ca.20 days following the cell infusion).

To reduce probability of MSC rejection, we are planning to use the cells from HLA-matched bone marrow donors (either related, or unrelated persons from available marrow donor registries). In case of their lack, we are suggesting to employ partially compatible, and, at least, incompatible donors. For HLA-incompatible donor/recipient pairs, the patients will be monitored for specific anti-HLA antibodies.

So far, there are no recommendations concerning choice of optimal dosage and regimens for MSC injections. In vast majority of clinical studies with MSC, the doses of 1-2x10⁶ cells/kg weight were used. Even higher cell doses were applied in some trials (8–10x10⁶ cells/kg), without any adverse effects [38, 59, 104]. The data about dependence of treatment efficiency on the cell dosage are rather controversial. A series of pharmacodynamic studies has shown that a dose of 1x10⁶ cells/kg exerted a weak, but statistically significant therapeu-
tic effect in a model of myocardial infarction in hamsters [89]. The same study yielded maximal therapeutic effects when using much higher cell doses (40×10^6 cells/kg). Meanwhile, some clinical results suggest that higher MSC doses are not more effective, when treating acute graft-versus-host disease [53]. Moreover, MSCs are rather large-sized cells which are prone to aggregation, thus, being able of blocking blood flow in small pulmonary vessels upon intravenous infusion. Therefore, the i/v infusions at abundant MSC concentrations may be accompanied by a risk of clinically significant embolization of the small blood vessels in lungs.

Due to proven clinical safety of i.v. MSC infusions at the dose scale of 1 to 10×10^6 cells/kg, a therapeutic window between the cell infusions, and time required for potential immune rejection, as well as economic considerations, our present study provides for two infusions of allogeneic MSCs per patient. The allogeneic MSCs should be applied on days +1 and +10 after a corrective osteotomy at a dose of 5×10^6 cells per 1 kg body weight.

Primary goal of our study is to assess safety of cryopreserved, ex vivo cultured allogeneic MSCs after intravenous infusion of the cells to the patients with osteogenesis imperfecta, evaluating acute infusion toxicity and immunogenicity (anti-HLA antibody production). Secondary goal is to study potential therapeutic effects of allogeneic MSC infusions in the patients by clinical parameters, e.g., decreased incidence of bone fractures within 2 years after MSC infusions; acceleration of linear bone growth rates, accelerated bone fragment consolidation, and increased mineral density of bone tissue, as well as laboratory markers of osteogenesis. This will be a prospective open-label single-center trial, Phase 1-2. Allogeneic MSCs will be isolated from the marrow of HLA-compatible related, or unrelated donors, in order to obtain ex vivo cultured MSCs. A total of 15 patients with osteogenesis imperfecta will be subjected to infusions of cryopreserved, ex vivo cultured allogeneic MSCs 24 h after a corrective osteotomy of femoral and/or tibial bone, at two doses of 5×10^6 cells/kg, with an interval of 10 days. Toxicity and potential efficiency of the treatment will be evaluated. A five-year observation period after MSC infusions is scheduled.

Conclusion

Multipotent mesenchymal stem cells (MSC) represent an adherent, easily cultured cell population from different sources which is recognized by specific markers and secretes a number of growth-promoting factors and cytokines.

Bone marrow-derived MSCs may differentiate to adipogenic or osteogenic direction. The fate and viability of infused MSCs is not studied in details, like as their in vivo differentiation abilities. The injected MSCs are shown to improve tissue repair processes and modulate adverse immune reactions, such as severe GVHD post-transplant.

Therefore, a novel protocol is proposed for treatment of osteogenesis imperfecta (OI), based on MSC injections performed during corrective bone plastics in the OI patients. A small group of cases should be observed for assessment of toxicity, immunogenicity and duration of potential therapeutic effects produced by MSC infusions.

Conflict of interests

No conflict of interests is declared.

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Potentially clinical applications of mesenchymal stromal stem cells in bone regeneration

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Resume

Bone marrow (BM) contains a population of so-called "multipotent mesenchymal stromal cells" (MSC). These adherent cells can be isolated and activated in simple nutritive media, and are capable of differentiating into adipocytes or bone cells. To date, there is no evidence of mass differentiation of MSC into hepatocytes, kidney cells or brain cells. At the same time, two possible clinical applications are considered: (1) as an immunomodulatory means in graft versus host disease (GVHD) and autoimmune diseases, or (2) as a potential source of growth-stimulating factors in specialized tissues. This heterogeneous population can sustain hematopoiesis by secreting growth factors, cytokines and other biologically active substances. After injection, MSC can migrate to injured tissues, thereby contributing to their regeneration. However, only a small fraction of MSC can reach bone marrow niches after intravenous infusion. Numerous experiments with MSC on different models of tissue injuries showed that their capacity to induce apoptosis, caused by hypoxic, chemical agents and other destructive factors. This protective effect is observed in various factors of the differentiative factors of the bone, for example, GM-CSF. In a large number of clinical studies, the safety of MSC is shown.

MSC. As clinical, as well as experimental studies, demonstrated a strong immunogenicity of allogeneic MSC when injected. To date, the presence of immunocompetent recipients. Since the time of transplantation, MSC have been shown to inhibit apoptosis in response to hypoxic, chemical agents and other destructive factors. This protective effect is mediated by a number of secreted growth factors, such as GM-CSF. In a number of clinical trials, the safety of MSC is shown. As clinical and experimental studies, have shown the weak immunogenicity of allogeneic MSC when they are injected into immunocompetent recipients. To date, several patients have been injected with cultured MSC after autologous transplantation of hematopoietic cellular therapy.

Keywords
Mesenchymal stem cells, biological distribution, secreted factors, proliferation in culture, clinical application, unspecific osteogenesis.