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How mesenchymal stem cells co-transplantation in hematopoietic stem cells can improve the engraftment in animal models

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Abstract

BACKGROUND

The bone marrow transplantation is a treatment that may be applied to both hematopoietic and non-hematopoietic diseases; nonetheless, it still comes with a number of challenges and limitations that contribute to treatment failure. Bearing this in mind, a possible way to increase the success rate of bone marrow transplantation would be the use of mesenchymal stem cells co-transplanted with the hematopoietic stem cells to improve the bone marrow niche and secrete molecules that enhance the hematopoietic engraftment.

AIM

To analyze the hematopoietic and mesenchymal stem cells characteristics and the several interactions through the co-transplantation in murine models.

METHODS

We searched for original articles indexed in PubMed and Scopus of the last decade that had used the hematopoietic and mesenchymal stem cells co-transplantation and *in vivo* bone marrow transplantation in animal models while evaluating the cell engraftment. Being excluded only *in vitro* studies or studies that involved graft *vs* host disease or other hematological diseases and publications in languages other than English. In PubMed were initially identified 555 articles and after selection, only 12 articles were chosen, meanwhile in Scopus, 2010 were identified, having 6 Left at the end of the screening and eligibility process.

RESULTS

Of the 2565 articles found in the databases, only 18 original studies attempt the eligibility criteria. Hematopoietic stem cells distribution by source showed the same proportions, the human umbilical cord blood or animal bone marrow, being administered mainly with a dose of 1×10^7 cells by intravenous or intrabone, already the mesenchymal stem cells had a high predominance of human donor with different sources (umbilical cord blood, bone marrow, tonsil, adipose tissue or fetal lung), using a lower dose, mainly 10^6 cells and ranging 10^4 to 1.5×10^7 cells, using almost the same routes. Almost all investigations conducted prior to the administration characterized the mesenchymal stem cells. The recipient used was mostly immunodeficient mouse submitted to low dose irradiation or chemotherapy. The main technique of engraftment in hematopoietic and mesenchymal stem cells co-transplantations evaluation was chimerism, followed by hematopoietic reconstitution and survival analysis. Besides the engraftment, homing and cellularity were also evaluated in some studies.

CONCLUSION

In conclusion, these preclinical findings found in this systematic review validate mesenchymal stem cells potential to enable hematopoietic stem cells engraftment *in vivo* in both xenogeneic and allogeneic hematopoietic cell transplantation animal models in the absence of toxicity.

Key Words: Mesenchymal stem cells; Hematopoietic stem cells; Bone marrow transplantation; Co-transplantation; Hematopoietic reconstitution; Engraftment

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Core Tip: The systematic review provided a current view on the characteristics of mesenchymal stem cells and hematopoietic stem cells co-transplantation to achieve successful engraftment and improve hematopoietic reconstitution, demonstrating a diversity in experimental designs and mesenchymal stem cells isolation and characterization protocols; however, the lack of standardization in mesenchymal stem cells use makes translation to clinical practice more difficult.

INTRODUCTION

Hematopoietic stem cell transplantation has saved many lives in individuals with severe hematologic diseases. However, the results need to be improved, and co-infusion of mesenchymal stem cells (MSC) could be the key to creating this therapy as a viable alternative. Bone marrow stromal cells in adult bone marrow (BM) were discovered by Friedestein, in 1968, an adherent, fibroblast-like population capable of reconstructing rudiments of bone *in vivo*[1]. These cells, which make up only 0.1 percent of mature BM cells, provide the supportive niche for hematopoietic stem cells (HSC)[2]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has proven a life-saving therapy for many malignant and nonmalignant hematological illnesses throughout the last few decades, allowing for complete blood cellular constituent recovery and the graft-versus-leukemia effect[3]. The HSC therapeutic impact depends on the successful engraftment of donor stem cells[4]. However, a number of significant issues might make transplantation difficult. Graft-versus-host disease (GVHD), which can be acute or

chronic, as well as disease recurrence and opportunistic bacterial, viral, or fungal infections, is one of the most serious and potentially fatal side effects of allo-HSCT. All of these conditions may cause serious morbidity and mortality in allo-HSCT recipients[3]. According to recent research[4,5], MSC are the primary cell implicated in HSC homing. Although the molecular interaction and/or grafting cytoarchitecture are yet unclear, more research is needed to assess the true efficacy of MSC co-transplantation in allo-HSCT.

The MSC have significant immunomodulatory effects on both the adaptive and innate immune systems as a result of this. MSC-modulated lymphocyte suppression appears to be mediated by paracrine processes such as secreted mediators (*e.g.*, transforming growth factor, hepatocyte growth factor, prostaglandin E2), participated in complex interactions with dendritic cells, and B-lymphocytes and T-lymphocytes cells, including T regulatory cells, killer cells, and a variety of T helper cells and metabolic activities [*e.g.*, indoleamine 2,3-dioxygenas (IDO)]. Furthermore, MSC have been shown to induce T cells to become polarized toward a regulatory phenotype, which may contribute to the reduction of inflammation, preventing GVHD, improving the bone marrow niche. MSC also increase the expression of many hematopoietic factors, inhibit apoptosis, allowing HSC to survive and proliferate in the stroma[6,7].

The MSC can directly affect HSC by releasing soluble compounds such as IDO, prostaglandin E2, nitric oxide, transforming growth factor, interferon-gamma, and interleukin 1, although the net interactions between cells are unknown. The other molecular process enhanced post co-transplantation (MSC/HSC) is C-X-C chemokine receptor type 4 (CXCR4) and the stromal cell-derived factor 1 (SDF-1) also known as C-X-C motif chemokine 12 (CXCL12), in recovery in murine models. The optimal self-renovation and proliferation of HSC were an impact by survival and homing into BM, as also induced mixed chimerism by MSC accelerate hematopoietic reconstitution[8], but these interactions are unclear. Several studies have been carried out to investigate the safety and/or efficacy of MSC co-infused in HSC recipients, but controversy persists, probably due to heterogeneous doses, routes and sources of MSC and HSC. To date, a

few literature reviews have summarized these conflicting results but have not yielded any encouraging findings.

As a result of the difficult in setting up the hematopoietic engraftment and MSC co-transplantation benefits, the goal of this review was to analyze the hematopoietic and mesenchymal stem cells characteristics and the several interactions through the co-transplantation in animal models.

MATERIALS AND METHODS

Search strategy

We searched for original articles that were indexed in PubMed and Scopus. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines were followed during all procedures. The following selected criteria of interest, keyword sequences [(Mesenchymal Stem Cell) AND (Hematopoietic Stem Cell) AND (Graft)], and boolean operators (DecS/MeSH) were used:

PubMed: ("Mesenchymal Stem Cells" [Title/Abstract] OR "Mesenchymal Stem Cell" [Title/Abstract] OR "Mesenchymal Stromal Cells" [Title/Abstract] OR "Mesenchymal Stromal Cell" [Title/Abstract] OR "Multipotent Stromal Cells" [Title/Abstract] OR "Multipotent Stromal Cell" [Title/Abstract] OR "Stromal Stem Cells" [Title/Abstract] OR "Stromal Stem Cell" [Title/Abstract] OR "Stromal Cells" [Title/Abstract] OR "Stromal Cell" [Title/Abstract]) AND ("Hematopoietic Stem Cells" [Title/Abstract] OR "Hematopoietic Stem Cell" [Title/Abstract] OR "Hematopoietic Progenitor Cells" [Title/Abstract] OR "Hematopoietic Progenitor Cell" [Title/Abstract] OR "Hematopoietic Cells" [Title/Abstract] OR "Hematopoietic Cell" [Title/Abstract]) AND ("Engraftment" [Title/Abstract] OR "Grafting" [Title/Abstract] OR "Graft"[Title/Abstract]).

Scopus: TITLE-ABS-KEY ("Mesenchymal Stem Cells") OR TITLE-ABS-KEY ("Mesenchymal Stem Cell") OR TITLE-ABS-KEY ("Mesenchymal Stromal Cells") OR

TITLE-ABS-KEY ("Mesenchymal Stromal Cell") OR TITLE-ABS-KEY ("Multipotent Stromal Cells") OR TITLE-ABS-KEY ("Multipotent Stromal Cell") OR TITLE-ABS-KEY ("Stromal Stem Cells") OR TITLE-ABS-KEY ("Stromal Stem Cell") OR TITLE-ABS-KEY ("Stromal Cells") OR TITLE-ABS-KEY ("Stromal Cell") AND TITLE-ABS-KEY ("Hematopoietic Stem Cells") OR TITLE-ABS-KEY ("Hematopoietic Stem Cell") OR TITLE-ABS-KEY ("Hematopoietic Progenitor Cells") OR TITLE-ABS-KEY ("Hematopoietic Progenitor Cell") OR TITLE-ABS-KEY ("Hematopoietic Cells") OR TITLE-ABS-KEY ("Hematopoietic Cell") AND TITLE-ABS-KEY (engraftment) OR TITLE-ABS-KEY (grafting) OR TITLE-ABS-KEY (graft).

Inclusion and exclusion criteria

This systematic review included only original articles written in English, published between 2011 and 2021, that had used (1) hematopoietic and mesenchymal stem cells co-transplantation; (2) *in vivo* bone marrow transplant in animal models; and (3) engraftment evaluation, factors involved in PICO criteria: (a) Problem: Inefficiency of HSC transplantation; (b) Intervention: MSC co-transplantation; (c) Comparison: HSC-only transplantation and associated with MSC; and (d) Outcome: Engraftment evaluation.

Reasons for excluding studies were as follows: (1) not original articles; (2) publications in languages other than English; (3) indexed articles published in more than one database (duplicates); (4) studies involving GVHD; (5) studies involving other diseases; and (6) studies with only *in vitro* results.

Data compilation and review

In this systematic review, seven of the authors (M.M.G.; F.A.O.; M.P.N.; L.P.N.; A.H.A.; O.F.M.D. and L.F.G.) independently and randomly selected (in pairs), revised, and evaluated the titles and abstracts of the publications identified by the search strategy in the databases cited above, and all potentially relevant publications were retrieved in full. These same reviewers evaluated the full-text articles to decide whether the

eligibility criteria were met. Discrepancies in study selection and data extraction between the two reviewers were discussed with a third reviewer and resolved.

M.M.G., F.A.O., M.P.N., and L.P.N. searched for mesenchymal stem cell characteristics; M.M.G., F.A.O., O.F.M.D., and A.H.A. searched for hematopoietic stem cell characteristics; M.M.G., F.A.O., M.P.N., and L.F.G. searched for mesenchymal stem cell and hematopoietic stem cell co-transplantation parameters, M.M.G., F.A.O., M.P.N., and L.P.N. searched for therapy evaluation. The analysis process and table plots were carried out by a full consensus of peers, respecting the distribution above.

Risk of bias

In cases of disagreement, a third, independent senior author decided to add or subtract data, decreasing the risk of bias. The final inclusion of studies into the systematic review was by agreement of all reviewers.

Data analysis

For all variables evaluated in the tables, the percentage distribution was used to characterize and illustrate the results.

RESULTS

Overview of the reviewed literature

We searched original articles published between January 2011 and December 2021, indexed in PubMed and Scopus, and a total of 2565 articles were found. Of the 555 articles identified in PubMed, 413 were excluded after screening (200 reviews, 212 studies in humans, and 1 published in another language), and 130 after eligibility assessment (27 *in vitro* studies, 42 GVHD studies, and 61 other hematological diseases), included only 12 studies of this database. Of the 2010 articles identified in Scopus, 1625 were excluded after screening (1140 not original articles, 390 studies in humans, 3 published in other languages, and 92 articles duplicated in PubMed search), and 379 after eligibility assessment (125 only *in vitro* study, 67 focus in GVHD study, and 187 in

other hematological diseases), being included 6 studies. As a result, 18 papers[8-25] met all the criteria for inclusion and exclusion in this systematic review (Figure 1).

Hematopoietic stem cell characteristics

In terms of HSC, half of the selected studies[8,10,15,16,18,20-22,25] used cells from human umbilical cord blood (UCB), while the other half[9,11-14,17,18,23,24] used cells from animal BM (Table 1). The study by Wu *et al*[20] reported the transplantation of the pool of human nucleated cells, meanwhile, Huang *et al*[10] and Lim *et al*[21] reported the use of a mononuclear cell pool without any cell selection process, already the other 33.3% of the selected studies used human cells CD34+ before transplantation. The majority of studies[9,11-14,18,23,24] used the pool of nucleated cells (44.4%) from animals, with the exception of the study by Fernández-García *et al*[17] (5.6%) that used cells lineage- Sca-1+ cKit+ (LSK).

The transplantation process was reported using mainly 1×10^7 cells as dose[9-11,21], ranging from 2.5×10^3[8] to 1×10^7 cells, with exception of the study by Kornblit *et al*[23] that used the cell dose of 1.8 to 5.3×10^8/kg in the Beagle dog with an administered volume of 50 mL. Four studies[8,10,15,25] compared two routes, Intravenous (IV) *vs* Intrabone (IB), but majority of studies used IV route (72.7%) with volume dose ranged 100 to 250 µL, followed by 22.2% of IB[8,10,15,25], being administered a volume between 10 to 20 µL.

Mesenchymal stem cell characteristics

The MSC used in co-transplantation of HSC had interesting features (Table 2). The main MSC source was human (61.1%) being extracted from difference sources, BM (16.7%), UCB (22.2%)[8,10,20-22], tonsil (11.1%)[9,11], adipose tissue (5.6%)[22], or fetal lung (5.6%), already the animal donor source of MSC (38.9%)[12-14,17,18,23,24], was 33.3% from BM[12-14,18,23,24], and 5.7% of adipose tissue[17]. Among animal donors, the study by Kornblit *et al*[23] used the Beagle Dog as an MSC donor, extracting the cell from BM.

For MSC characterization, the selected studies reported mainly the following cluster of differentiation (CD) surface markers, the negative expression of CD45 (66.7%)[9-12,14,15,17,18,20-24], CD34 (55.6%)[9-11,15-18,20-23], and CD14 or CD11b (38.9%)[9,11,20-22] for both humans and animals source cells, and the positive expression of the CD73 (64.3%)[9,11,13,15,17,20-22,25], CD105 (64.3%)[9-11,15,17,20-22,25], CD90 (57.1%), and CD44 (42.9%) markers.

Most of the selected studies (61.1%)[9-12,14,15,17,18,21,23,25] reported cell uses with a passage between P1 to P8, mainly in the low passage (P3 and P4). Only 33.3% of the studies reported some type of cell modification such as the use of donors cell deficient in type 2 nitric oxide ($Nos2^{-/-}$), which is related to the immunosuppressed activity of MSC and to the differentiation and expansion of MSC and myeloid cells[12]; metalloproteinase 3 knockdown (MMP3-knockdown), a metalloproteinase that degrades proteins from the extracellular matrix and activate others matrix metalloproteinase[9], facilitating the homing; the overexpression of CXCR4, which is essential for the homing and maintenance of HSC in BM niches; epidermal growth factor (EGF), involved in the HSC long term recover and improve the mice survival rate after facilitating the homing[8]; SDF-1, a chemokine that perform an important role in the HSC homing to the BM; Homeobox B4 (HOXB4) that is involved in the HSC stimulation and self-renovation[19]; or soluble granulocyte colony-stimulating factor decoy receptor (solG-CSFR) that is a receptor for the granulocyte colony-stimulating factor (G-CSF), a cytokine known for inducing the cellular mobilization that is increased in the mice BM shortly after total body irradiation (TBI)[24]. About the MSC transplantation, the cell dose used was mainly around 10^6 (25%)[9,10,16,17,20,22], ranging from 10^4[13] to 1.5×10^7 cells[24]; 66.7% of the selected studies used the IV route, being administered a volume ranged 100 to 250 µL, meanwhile the other 23.8%[8,10,13,15,25] administered by IB, using volume ranged 3 to 20 µL, and 4.8%[24] of the studies used intraperitoneal (IP) and did not report the volume administered. In the study by Kornblit *et al*[23], the cell dose (4.8 to 10×10^8/kg) was greater due to the use of dogs as a host, using 50 mL as a volume for administration.

Bone marrow transplant model

To achieve a supported and quantitative HSC engraftment after bone marrow transplantation (BMT), is necessary to condition the animal with irradiation or chemotherapeutic before then transplantation. Bearing this in mind, 88.9% of the selected study (Table 3) used the TBI with different types of radioactive sources (18.8% by Caesium-137[15,21,25], 31.3% by Cobalt-60, and 50% did not specify the source[8,12-14,16,17,20,22]), and 11.1%[9,11] used chemotherapy with Busulfan (Bu) and cyclophosphamide (Cy). The dose of conditioning (intensity and frequency) varies depending on the resistance of the host animal, in the C57BL/6 mice were reported high doses (from 5[17] to 9 Gy[14]), in BALB/c mice (7.5 Gy of Co^{60}[18] or from 20 to 25 mg/kg/d of Bu associated with 100 mg/kg/d of Cy[9,11]), already in NOD/SCID mice was reported low irradiation doses, ranging from 2.5[8,15,21] to 3.5 Gy[16,19,20]. 61.1% of the xenogeneic transplants were performed on NOD/SCID mice using human HSC and MSC, and the BALB/c mice also received human MSC[9,11]. In the other studies (38.9%)[12-14,17,18,23,24] were performed allogeneic transplants in mice (BALB/c, C57BL/6, or FVB Insulin-GFP), and in the study by Kornblit *et al*[23] was used as recipient and donor cells, the Beagle dog. The experimental groups involved different conditions of analyses, but only 4 studies (22.2%) included the control group (untreated condition), while in the other studies the basal reference was the group that used only HSC transplantation to compare with other conditions.

The HSC and MSC Co-transplantion evaluation

The primary goal of the studies included in this review was to assess the therapeutic efficacy of HSC and MSC co-transplantation using various MSC sources, cell alterations, and niche environment as described in Table 4. The chimerism analysis by flow cytometry was the main approach employed (83.3%)[8-10,12-20,22,24,25] for this evaluation, followed by 44.4% hematopoietic reconstruction analysis by blood count or flow cytometry or immunohistochemistry[9,12-14,18,19,21,23], 22.2% homing analysis by flow cytometry or images[9,10,17,24], survival (Kaplan-Meier estimator)[9-11,19], and cellularity

analyses (HE and Wright staining)[9,18,19,21], and in low frequency (5.6%) platelet reconstruction by flow cytometry[16], expression of hematopoietic cytokines by immunohistochemistry[10], and Thymus regeneration by different techniques (volumetry, histological and immunohistochemistry analyses)[11].

The chimerism was assessed throughout a period of time extending from 7 to 112 d, with the analysis becoming more visible around the 12th and 42nd days, and showed that the number of donor cells in the recipients was higher in co-transplantation with MSC than in the HSC-only transplantation, and the co-transplantation had a lower chimerism than control group[15]. However, three studies[12,18,23] did not show any difference in the use of co-transplantation, comparing the groups HSC *vs* HSC+MSC. The most used marker for chimerism analysis was the human CD45^{+} (60%)[8,10,15,16,19,20,22,24,25], while other studies used murine cell markers such as CD45.1^{+}, H-2^{b} and H-2^{d} (33,3%)[9,12,14,17,18] and a single study used green fluorescent protein (GFP) expression by HSC for flow cytometry analysis[13].

The hematopoietic reconstruction was evaluated up to 49 d after co-transplantation, with the exception of one study[23] that evaluated at 100 d. However, significant results were found between 7 and 14 d after transplantation, after this period the results did not show relevant differences in the group comparisons about the number of circulating white blood cells, except for the study by Kim *et al*[14] that reported difference only the group of co-transplantations of HSC with MSC under stimulatory condition.

The cell homing was evaluated within 24 h after transplantation (2, 4, 18, 24 h) and the MSC, modified or not, showed an increase and improve the cell homing, facilitating hematopoietic reconstitution[9,10,17,24].

The survival analysis was performed mainly around 24 and 40 d, showing that the HSC and MSC co-transplantation group had higher survival in comparison with the HSC group, and in selected studies[9,19] that used the MSC modification (for example, HSC+SDF1-HOXB4-MSC) can improve even more this survival during co-transplantation, in comparison to other groups.

The cellularity analysis was performed between 7 and 56 d (mainly 14 and 28 d), showed a significant increase in the groups that used the MSC and HSC co-transplantations, with or without MSC modifications[9,18,19,21].

Some of the specific analyses provided by the selected studies revealed, for example, that the use of HSC expanded with thrombopoietin (Ex/TPO-HSC) resulted in an increased of the platelet number only in short time (14 d after transplantation), but the use of MSC had influence on platelet production in short and long term (14 and 42 d)[16]. Other selected studies[11] revealed, using different techniques of evaluation (3, 10, and 40 d after cell transplantation), that the co-transplantation improve the thymus regeneration, as well as increase the expression of hematopoietic cytokines such as the vascular endothelial growth factor (VEGF-A), osteopontin (OPN), and SDF-1 independently of route (at 42 d after UCB-MSC and HSC transplantation). Overall, 72.2% of the selected studies[8-10,13-17,19,20,22,24,25] reported an improvement when HSC and MSC are co-transplanted. A meaningful improvement can be observed when MSC expressed platelet derived growth factor subunit B (PDGFB)[8], SDF1-HOXB4, CXCR4[18] are co-transplanted, or with the co-administrations of the human parathyroid hormone (hPTH)[21] in comparison to HSC and MSC co-transplantation. In the study by Kim *et al*[14] there was only improvement in the engraftment when the MSC were previously cultured with a stimulating serum. In the study by Fortin *et al*[24], solG-CSFR-MSC co-transplantation improved homing and accelerated hematopoietic reconstitution, but not engraftment, when compared with HSC+MSC co-transplantation.

Figure 2 summarizes the key findings of this systematic review on the characterization of HSC and MSC (donor and source percentile distribution), the importance of evaluating MSC before their administration using surface markers (positive and negative expressions), the differences in doses (HSC: orange bars and MSC - pink bars of Figure 2 histograms) and routes of each cell in co-transplantation (IV, IB, IP), and the main techniques for evaluating the therapy's success and improvement in the grafting process, shown by spider chart.

DISCUSSION

Many hematological diseases can be cured with allo-HSCT and optimizing the homing and overall survival process is a critical step. The main goal of this systematic review was to search at preclinical studies of MSC and HSC co-transplantation, demonstrating several aspects of the MSC and HSC characteristics and transplantation process, as well as showing molecular and/or structural synergism aspects of co-transplantation that result in complete successful engraftment.

After searching original articles published between January 2011 and December 2021, indexed in PubMed and Scopus, the current systematic review examined 2565 preclinical studies of evaluation of the use of MSC in HSC engraftment in the animal model and included only 18 studies. Most of the papers were produced by Asian researchers and published between 2013 and 2016, with assistance from a number of countries, most notably the United States of America. A recent systematic review that included meta-analysis of clinical trials on the same topic found 19 studies (searching in 6 databases), 10 of which (52%) were developed in China, with part of them being published in Chinese and the other 13 published in the English language[4]. According to this evaluation and previous investigations by our group[4,26,27], there is a considerable concentration of evidence generation in this field of knowledge by Chinese researchers groups, with the most recent studies on HSC and MSC co-transplantation focusing primarily on models of hematological diseases, particularly GVHD, with some meta-analyses of clinical trials published on the topic[28-31], approaches that were excluded from our review based on pre-determined criteria.

Most of the studies found that the graft improved when comparing HSC and MSC co-transplantation to HSC-only transplantation. The HSC and MSC co-transplantation has previously been shown to promote engraftment, chimerism, and homing in a variety of species including monkeys[32], sheep[33], mice[34], and humans[35]. Despite the fact that the majority of studies have yielded excellent results, there are still challenges to be solved, such as the heterogeneity of MSC sources, the volume of cells administered, the optimum route of administration, and the safety of MSC transplantation[4]. The

improvement of the self-renovation and proliferation of HSC in BM is a critical step for the success of engraftment or transplantation as therapy for many hematopoietic and immune system disorders[5]. This condition is influenced by a several number of factors, the most important of them are the characteristics of the engrafted HSC[27]. HSC from human UCB were used in half of the studies in this review, while HSC from animal BM were used in the other half. Two recent reviews found the same aspect and percentage of HSC source distributions[36,37], showing that the source of human HSC has an impact on a variety of clinical outcomes, particularly survival and homing, and the BM HSC have better survival and homing than UCB HSC[38]. A recent study found that the cell source chosen is influenced by a variety of patient and diagnosis-related characteristics, as well as the availability of appropriately-matched donors, leaving the question of which cell source is superior or has more benefits unanswered[39]. The recent systematic review of clinical trials[40] also reported the use of the BM HSC as mainly source by IV transplantation. The study by Wu *et al*[20] reported the transplantation of a pool of human nucleated cells as the majority of the selected studies included in this review, meanwhile, Huang *et al*[10] and Lim *et al*[21] reported the use of a mononuclear cell pool without any cell selection process, and Fernández-García *et al*[17] that used LSK cells.

The most of selected studies[8-16,18-22,24,25] showed success in the ability of myeloid cells to expand and of MSC to accelerate hematopoietic regeneration or self-renovation independently of the dose used, ranging from 2.5×10^3 to 1×10^7 cells, but the majority of studies used the dose of 1×10^7 cells in around of 200 µL of volume administration, with exception the study by Kornblit *et al*[23] that used the cell dose of 1.8 to 5.3×10^8/kg in the Beagle dog in 50 mL of volume. However, the study by Fernández-García *et al*[17] showed that the improvement of HSC engraft is MSC dose-dependent, mainly in later stage. However, Park's study found an adipose tissue derived mesenchymal stem cells (AT-MSC) dose-dependent hematopoietic engraftment effect, which we also could see in our autologous transplantation model. Fernández-García *et al*[17] used one of the lowest doses reported by selected studies in our review to confirm an efficient immunomodulatory effect of MSC in the BM niche. A recent systematic review of

clinical trials[4] found no association between allo-HSC dose and better outcomes, but another review found that patients who were infused with higher dose HSC had better survival rates than those who were infused with lower dose HSC. These studies also found a stronger link between the route, dose, and other MSC features.

The variety of HSC delivery modalities investigated (IV and IB,) aims to improve therapeutic outcomes by enhancing homing and engraftment. Only 22.2% of studies[8,10,15,25] compared the HSC administration routes (IV *vs* IB) in the HSC co-transplantation with MSC, however, the IV route was the most common in all selected studies (72.7%), and in the comparison between routes, the IB *via* showed better specific HSC graft results when associated with some MSC modifications[8,10,15]. Curiously, the studies normally use the same route for HSC and MSC administrations, but the study of Abbuehl *et al*[13] adopted the IB *via* for MSC and the IV *via* for HSC. Some preclinical studies have shown that direct IB marrow injection of MSC can enhance the engraftment of cord blood cells more than the IV injection, however, the MSC administrated by IV route were retained, mainly in the lung. In clinical studies, the IB marrow injection of MSC is shown safe.

The MSC were administered mainly by IV route (66.7%), but the studies that compare the IV and IB routes[10,15,25], showed that there is an improvement in the HSC outcomes in IB route[8], the Futrega *et al*[15] reported better results when administration both cells were by the IB route, increasing the HSC number in the local of administration, however, did not improve the systemic engraftment, and the study by Huang *et al*[10] also reported the improvement of survival when the cells were administration by in IB route was slightly higher in comparison to the IV route. A single study[24]also used the IP route, with 1.5×10^7 cells administered per animal. However, it has already been demonstrated that MSC injected into the peritoneum aggregate the cells with macrophages in the peritoneal cavity, limiting the amount of viable MSC available for therapy[41]. The average number of cells given to the animals that received MSC by IV was higher than that given to animals who received MSC *via* the IB route. This is probably due to the medullary cavity's small capacity, which allows a greater number

of MSC to be delivered systematically. Despite the fact that the IV route does not have the same spatial limitations as the IB route, it is still vital to pay attention to the number of cells that will be infused, since large doses can cause thrombolysis and threaten the animal's survival[42]. The study by van der Garde *et al*[16] reported deaths at the time of administration, probably due to the increased size of MSC, which ended up being held in the lungs due to a phenomenon known as the lung barrier. Pneumopulmonary edema was observed 9 d following MSC injection in dog research by Kornblit *et al*[23].

Before analyzing the routes and doses for the MSC transplantation, it is extremely important to characterize these cells, and it was carried out in 78% of studies, only two studies did not report this analysis, using MSC from animal BM[18], and human UCB[8], and the study by Kornblit *et al*[23] used the PCR technique for this goal. Among the negatively expressed markers, the most used for MSC characterization were CD34, CD45, CD14 or CD11b, and HLA-DR, and the common markers for human and animal MSC were CD34, CD45, CD80, and CD31. The human MSC used a greater number of negative markers in this characterization, mainly for tonsil and umbilical cord blood cell sources. As reported in studies that analyzed the mouse MSC characterization and others that focus on human MSC characterization, as well as the minimal criteria for defining multipotent MSC[43,44] the following positive markers, CD105, CD90, CD44, CD73, CD29, were common for human and animal MSC, with the exception of human MSC CD95, CD75, and animal MSC, Sca-1, PDGFR, CD106, CD144, CD146, and CD13. As a result, MSC from animals had more positive indicators than human MSC, primarily for adipose tissue. Therefore, MSC from animals had more positive markers than human MSC.

Besides the characterization of MSC, most studies[8,10,15,16,19-22,25] that aimed to evaluate the efficacy of HSC and MSC co-transplantation used the humanized mice models. This model involves the transplantation of human cells into an immunodeficient animal. This technique allows for the examination of a variety of disorders that would not be viable in humans, as well as a step forward in the development of clinical trials[45,46]. Humanized mice have been used for decades to better understand the mechanics of

BMT, including HSC homing and grafting[47-50]. Despite the increased usage of MSC in clinical trials in recent research, the findings are still inconsistent.

Chimerism analysis in recipient animals, which is the assessment of the frequency of donor cells in recipients achieved by particular biomarkers of human blood cells such as $CD45^+$[8,10,15,16,19,20,22,24,25] was the main method of evaluating the graft found in our study. The chimerism was assessed throughout a period of time extending from 7 to 112 d and three of the 15 studies looking at chimerism indicated that co-transplanting HSC with MSC alone did not benefit the graft[12,18,23], whereas the others found that when recipient mice were co-transplanted with MSC, the frequency of donor cells increased. It's also interesting to note that several studies modified the MSC to see how protein expression in the transplant affected the results.

In the cell homing analysis[9,10,17,24] occurred within 24 h after cell transplantation (2, 4, 18, 24 h), the co-transplantation of HSC and MSC increased the cell homing, facilitating hematopoietic reconstitution after HSC engraftment. However, the study by Fernández-García *et al*[17] study, showed that the HSC and MSC co-infusion of not only BM-derived, but also Ad-MSC with low numbers of HSC significantly enhanced short- and long-term hematopoietic reconstitution in an autologous transplant setting in mice. The study by Lee *et al*[9] observed a higher homing independently of the expression of MMP3. Already, in the Fortin *et al*[24] study only MSC with the presence of solG-CSFR increased the homing. The ability of MSC to homing to target after the infusion is one of the most essential characteristics of their efficacy in tissue regeneration[51]. Through the production of paracrine mediators, it may be possible to reestablish the BM microenvironment that has been disrupted by the conditioning regimen, resulting in enhanced homing[4]. MSC improved hematopoiesis by increasing $CD123^+$ HSC expression, implying myeloid differentiation[52].

Hematopoietic reconstitution is the most important outcome after allo-HSCT, with most studies[9,12-14,18,19,21,23] a fast increase in blood cells or a high number of leukocytes after 14 d of co-engraftment MSC/HSC. The co-infusion of HSC with MSC overexpressing CXCR4 or SDF-1/HOXB4 enhanced post-transplant hematopoietic

recovery in murine models[52]. Extracellular vesicles, including microvesicles and exosomes, have been proven to represent a key conduit of intercellular communication between MSC and HSC, leading to improved hematological recovery Furthermore, the hematopoietic system's regenerative properties may apply to other tissues. MSC-educated myeloid cells exhibit a molecular and functional profile that is similar to that of resident macrophages, which have been implicated in tissue healing in other organs[53].

In addition to HSC and MSC co-transplantation to increase engraftment. MSC have been manipulated in some experiments to express chemicals, promote migration, or improve the hematopoietic niche in which HSC can grow. The animal's pre-conditioning causes destruction in the BM microenvironment; keeping this in mind, it is also established that niche conditions have a direct impact on the efficiency of hematopoietic recovery. Growth factor expression is involved in the restoration of the BM microenvironment. The study by Yin *et al*[8], growth factors [EGF, fibroblast growth factor 2 (FGF2), and PDGFB] were overexpressed in MSC, and these factors have a beneficial effect on niche regeneration following irradiation. When compared to the group that simply received cells, the group that got PDGFB-MSC had a higher frequency of $CD45^+$ and $CD34^+$ cells.

Among MSC modifications used in a few studies[8,9,12,18,19,24]was verified that MSC genetically modified for Nos2$^{-/-}$, did not show the ability to differentiate and expand myeloid cells and macrophages when compared to the HSC and MSC co-transplantation group[12]; BM-MSC with recombinant adenovirus expressing an SDF-1/HOXB4 fusion gene co-transplanted with human cord blood $CD34^+$ HSC (CB-HSC) showed beneficial effects on hematopoietic recovery and survival in lethally irradiated mice, as also significantly increase HSC growth *in vitro* and engraftment *in vivo*; MSC overexpressing solG-CSFR improved the homing, but did not accelerate hematopoietic reconstitution in mice. The increase in the level of G-CSF post-irradiation can have a long-lasting impact on homing, possibly through the effects of G-CSF on osteoblasts homeostasis and the SDF-1a/CXCR4 axis[24]; already in the study by Yin *et al*[8], only the

PDGFB-MSC showed significant results in comparison to other cell modifications (EGF, and FGF2), enhancing the MSC survival and expansion after transplantation, improving the human HSC engraftment in immunodeficient mice, and transplanted human HSC self-renewal in secondary transplantations, and the knockdown by siRNA of MMP3 in MSC can influence negatively the engraftment and the homing of MSC and HSC[9].

The main limitation of this study is that few studies have looked at how MSC enhance HSC engraftment, like the evaluation of homing promotion by mesenchymal secretion of MMP3, PDGFB, and solG-CSFR, as well as the interaction of the SDF-1/CXCR4 axis and the binding of the HOXB4 in HSC self-renewal. Only one study[10] looked at variables like VEGF-A, which acts as an anti-apoptotic, and OPN, which is linked to the ability of HSC to regenerate and the pool of progenitors in the bone marrow. The mechanisms involved in co-transplantation have been largely ignored in most investigations, and although it is widely assumed that both cell-to-cell interaction and release of soluble substances play a role, the mechanisms by which MSC perform their roles have not been explained clearly, being a potential source of bias in the studies outcome's interpretations included in this review. Another limiting factor that prevented more conclusive results on co-transplantation from being found was the use of a wide variety of experimental designs in the included studies, primarily regarding the source of the cells, the dose administered, and the heterogeneity of the protocols used for isolation and characterization of MSC. Some studies[54-56] that reported the co-transplantation performed a co-culture of both MSC and HSC and administrated only HSC in the host animals, this methodology bias was excluded during the selection process. The standardization of this, particularly with regard to MSC, is a critical step toward the clinical adoption of co-transplantation[57]. This systematic review did not include the co-transplantation of MSC and HSC in hematological disease models to assess the immunosuppressive role they play, particularly in the control of GVHD, which is one of the primary applications of MSC in BMT.

Notwithstanding these methodological limitations in the preclinical research, clinical trials already reported significant results related to the increase of the engraftment and

survival rate through the HSC and MSC co-transplantation. The recent systematic reviews of clinical trials[4,40,58] evidenced that the more homogeneous the MSC are in terms of the donor, source, extraction way, culture, and other aspects, the better is their efficacy and potentially, the less treatment dose required, and therefore the less likely it is to cause adverse events. These clinical trial reviews showed higher use of allogeneic MSC co-transplantation in allo-HSC, in phase II, or autogenic sources of MSC in phases I and II, during co-transplantation of HSC and MSC increase the survival rate in clinical trials than analysis other animals' studies. However, many of these studies also reported some difficulties like the ones found in this revision, such as the wide variety of biological characteristics of stem cells and the MSC source[38], which makes it difficult to understand the real mechanism responsible for improving the engraftment and decreasing the self-rejection, factors that should be initially elucidated in pre-clinical analysis to facilitate the prospective clinical results.

Despite the difficulties mentioned above in the development of MSC as a therapy, recent scientific discoveries highlight the unrealized therapeutic potential of MSC and suggested that MSC will become a key component of the hematological therapy armamentarium[3]. The first step for this is happening with elucidate the complete mechanism of specific therapeutic activity must be understood. The MSC have a wide range of immune-modulating properties, but it's unclear whether they use all of them in all circumstances. Such scientific understanding will help in the creation of much-needed clinically applicable potency assays, as well as tactics to boost MSC potency, such as genetically editing[5] MSC, and aims to improve manufacturing protocols, all of which are key components of long-term success. The second hurdle is that investigators must comprehend better the timing of MSC activity in phase II and III clinical trials[4,40].

CONCLUSION

In conclusion, these preclinical findings found in this systematic review validate MSC's potential to enable HSC engraftment *in vivo* in both xenogeneic and allogeneic hematopoietic cell transplantation animal models in the absence of toxicity. Some MSC

modifications using in the co-transplantation showed an even greater benefit of HSC engraftment, as also in accelerating hematopoietic reconstruction in preclinical studies. However, the best cells characteristics for this application is still inconclusive due to the diversity and heterogeneity of the studies, but their potential can be detectable in malignant with leukemias and nonmalignant hematological disorders with anemies and others hemoglobinopathies.

ARTICLE HIGHLIGHTS

Research background

Although bone marrow transplantation (BMT) may be applied to the treatment of hematological and non-hematological diseases, this treatment still presents a series of difficulties and obstacles that corroborate to the treatment failure.

Research motivation

The motivation to study the use of mesenchymal stem cells (MSC) in hematopoietic stem cells (HSC) transplantation is that the use of both cells at once may increase the success rate of BMT.

Research objectives

The purpose of this systematic review was to investigate the characteristics of HSC and MSC, as well as their various interactions in murine models of co-transplantation.

Research methods

A systematic review was conducted in the PubMed and Scopus databases, looking for original articles from the last decade that used hematopoietic and MSC co-transplantation, as well as *in vivo* BMT in animal models, excluding studies involving graft-versus-host disease or other diseases.

Research results

Only 18 of 2565 articles found in the databases attempted the eligibility criteria. Regarding the cell characteristics used in the selected studies, mostly used MSC from humans of different sources, characterized before administration, using a lower dose than HSC, but by similar routes. HSC were half from the human umbilical cord blood and another half from animal BM and the recipient was a mainly immunodeficient mouse irradiated. The co-transplantation was evaluated mainly by chimerism followed by hematopoietic reconstruction, showing HSC engraft improvement with the conjunction MSC implantation.

Research conclusions

Our review evidenced that preclinical findings found in this systematic review validate MSC's potential to enable HSC engraftment *in vivo* in both xenogeneic and allogeneic hematopoietic cell transplantation animal models.

Research perspectives

The use of HSC in BMT shows promise in the improvement of the engraftment in animal models, however, still is necessary the MSC standardization to evaluate the real potential of the therapy in humans.

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