Endocrinology, Diabetes & Metabolism CASE REPORTS

S M Becerra-Bayona and others | Cell therapy to treat diabetic foot ulcers

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Mesenchymal stem cells derivatives as a novel and potential therapeutic approach to treat diabetic foot ulcers

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Summary

Diabetic foot ulcer morbidity and mortality are dramatically increasing worldwide, reinforcing the urgency to propose more effective interventions to treat such a devastating condition. Previously, using a diabetic mouse model, we demonstrated that administration of bone marrow mesenchymal stem cells derivatives is more effective than the use of bone marrow mesenchymal stem cells alone. Here, we used the aforementioned treatments on three patients with grade 2 diabetic foot ulcers and assessed their beneficial effects, relative to the conventional approach. In the present study, two doses of cell derivatives, one dose of mesenchymal stem cells or one dose of vehicle (saline solution with 5% of human albumin), were intradermally injected around wounds. Wound healing process and changes on re-epithelialization were macroscopically evaluated until complete closure of the ulcers. All ulcers were simultaneously treated with conventional treatment (PolyMen® dressing). Patients treated with either cell derivatives or mesenchymal stem cells achieved higher percentages of wound closure in shorter times, relative to the patient treated with the conventional treatment. The cell derivative and mesenchymal stem cells approaches resulted in complete wound closure and enhanced skin regeneration at some point between days 35 and 42, although no differences between these two treatments were observed. Moreover, wounds treated with the conventional treatment healed after 161 days. Intradermal administration of cell derivatives improved wound healing to a similar extent as mesenchymal stem cells. Thus, our results suggest that mesenchymal stem cell derivatives may serve as a novel and potential therapeutic approach to treat diabetic foot ulcers.

Learning points:

- In diabetic mouse models, the administration of mesenchymal stem cells derivatives have been demonstrated to be more effective than the use of marrow mesenchymal stem cells alone.
- Mesenchymal stem cells have been explored as an attractive therapeutic option to treat non-healing ulcers.
- Mesenchymal stem cells derivatives accelerate the re-epithelialization on diabetic foot ulcers.

Background

Currently, diabetes represents one of the world's major public health issues, which poses a significant socioeconomic burden to health systems (1). According to the World Health Organization, approximately 8.8% of the world's population are affected by this disorder; although, based on estimations, this number could increase to 48% by 2045 (1). Diabetes-related foot complications have been identified as the most common isolated cause of morbidity

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among diabetic patients, as well as the leading cause of amputation (1). In fact, the effects of hyperglycemia on the nervous system can prevent asymptomatic patients from noticing diabetic foot ulcers (DFUs), until such lesions are in a non-healing chronic state. Currently, available treatments for DFUs involve debridement, dressings, and antibiotics. Nevertheless, around 50% of DFUs are refractory to these therapies, even when using promising techniques such as chemicals, dressings and skin grafts (2). Therefore, new strategies to stimulate skin regeneration may provide a novel therapeutic approach to reduce non-healing ulcer disease (2).

In this context, mesenchymal stem cells (MSCs) have been explored as an attractive therapeutic option to treat non-healing ulcers (3). MSCs offer outstanding advantages over other stem cell populations, due to their multipotent nature, as well as their ability to home and engraft into damaged tissues, release trophic factors, promote neovascularization, manage oxidative stress and modulate an anti-inflammatory response (3). MSCs are obtained from live and cadaveric donors (4) and can be both efficiently expanded *ex vivo* and transplanted without previous conditioning of the patients, as opposed to total bone marrow or hematopoietic stem cell transplantation. Moreover, we have previously demonstrated, using a diabetic mouse model, that MSC acellular derivatives could be potentially used as an effective therapeutic tool given that they favored wound closure kinetics and reduced severe leukocyte infiltration (5). Also, the use of these acellular derivatives increased the formation of granulation tissue and remodeled the orientation of deposited collagen (5, 6). This therapeutic effect is attributed to the presence of pleiotropic bioactive molecules in the acellular derivatives produced by MSCs, which appeared to initiate and improve the wound healing process as well as facilitate the host response to tissue repair (5, 6). Considering these findings, three patients with grade 2 DFUs (classification system for research purposes described by the International Working Group of the Diabetic Foot) (7) were treated in order to assess whether the local administration of allogenic human bone marrow MSC derivatives (allo-hBM-MSCDs) has more beneficial effects in DFU healing than both allogenic human bone marrow derived MSCs (allo-hBM-MSCs) and a conventional treatment (PolyMen® dressing).

Case presentation

All participants met the inclusion criteria (Table 1) and they had an appropriate metabolic control of the disorder with 7–8% glycosylated hemoglobin values before and during the study. In addition, patient characteristics such as co-morbidities and concomitant medications are described in Table 2 and wound baseline characteristics are presented in Table 3.

Case 1 was a 71-year-old male, with a lesion on his right foot sole, which had a surface area of 4.05 cm^2 (Fig. 1A) and had been unhealed for 2 months. Similarly, case 2 (a 59-year-old male) presented a lesion on his right foot sole with a surface area of 4.42 cm^2 (Fig. 1A), which had been unhealed for 2 months. In contrast, case 3, a 58-year-old male, presented two interconnected lesions on his left foot sole that had been unhealed for 1 month. The surface areas of the left and right ulcers were 5.3 cm^2 and 3.7 cm^2, respectively. Both lesions were treated (Fig. 1A and Supplementary Figs 1, 2, see section on supplementary materials given at the end of this article).

Treatment

The study was conducted in accordance with the Declaration of Helsinki. All protocols were approved by the Research Ethics Committee at Fundación Oftalmológica de Santander – FOSCAL, Colombia (Act. No. 46/May 20th, 2016). Prior to MSC isolation and the start of wound treatment, informed consents were obtained from both, bone marrow donors and study participants, respectively.

Patients were randomly assigned to receive one of the following treatments (Supplementary Table A1):

(i) Conventional treatment, which consisted on intradermally applying 1 mL of vehicle (saline solution with 5% human albumin) at four peripheral

Table 1 Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Adult male or female, 40 years of age or over (until 80 years old) • Diagnosis of diabetes • Presence of grade 2 DFU • Surface area between 0.5 and 5.5 cm^2	• Condition of cancer • Symptomatic coronary disease • Presence of osteomyelitis • Diagnosis of brain or hematologic disorders • Use of immunosuppressive or cytotoxic drugs • Any acute systemic infectious disease process

Table 2 Patient characteristics.

Case number	Co-morbities	Concomitant medications
1	Diabetic retinopathy, arterial hypertension, primary hypothyroidism and chronic occlusive arterial disease	Losartan, levothyroxine sodium, atorvastatin, acetylsalicylic acid, metformin, insulin glulisine and insulin glargine
2	Chronic occlusive arterial disease and arterial hypertension	Atorvastatin, acetylsalicylic acid, Losartan, metformin, insulin glulisine, insulin glargine and acetaminophen
3	Diabetic neuropathy, chronic occlusive arterial disease, coronary artery disease with history of myocardial revascularization and chronic venous insufficiency	Metformin, insulin glulisine, insulin glargine, atorvastatin, carvelidol, acetylsalicylic acid, thiamine, diclofenac and acetaminophen

sites of the ulcer (0.25 mL of vehicle on each site of the lesion) in one dose at day 0. Therefore, this patient was only treated with the wound dressing based on PolyMen® (S2Medical AB).

(ii) One million of allo-hBM-MSCs were obtained from a healthy 27-year-old female donor, unrelated to the patient. Surface markers of allo-hBM-MSCs were evaluated and results were positive for CD73, CD90, and CD105 and negative for CD45, CD34, CD11b, and HLA-DR (data not shown). These cells were intradermally administered around the wound area at four sites (2.5×10^5 cells on each site of the lesion) in one dose at day 0.

(iii) One milliliter of allo-hBM-MSCDs were obtained from culturing the allo-hBM-MSCs. Similar to the other two treatments, allo-hBM-MSCDs were intradermally administered at four peripheral points of the lesion (0.25 mL of allo-hBM-MSCDs on each site of the lesion) in one dose at day 0. A second dose was repeated at day 7.

Follow-up

The follow-up visits were at days 1, 3, 7 and, after this, every week in order to evaluate the percentage of wound extent and support the healing process, which consisted on treating the ulcers with a wound dressing (PolyMen®).

During each visit, wound size (area, volume, mean depth and max depth) was accurately measured using 3D laser technology (SilhouetteStar camera). Time elapsed to complete wound closure was defined as the time in which the wound bed became completely re-epithelialized and filled with new tissue. The percentage of wound extent was calculated using the equation: ((surface area of actual wound/surface area of original wound) × 100).

In addition, macroscopic changes on tissue (re-epithelialization) were evaluated at days 1, 3 and, after this, every week.

Investigation

As shown in Fig. 1A, wound closure started to be noticed after 3 days of treatment with either allo-hBM-MSCDs or allo-hBM-MSCs, as compared to the conventional treatment, for which wound closure was first observed at day 7. In fact, the percentage of wound closure in the allo-hBM-MSCD and allo-hBM-MSC treated patients was higher than that for the patient treated with the conventional approach (Fig. 1B). Specifically, after 7 days of treatment, the allo-hBM-MSCD and allo-hBM-MSC patients achieved a 27.84% and 35.89% decrease in wound surface area, respectively; instead, the patient treated with the conventional approach only achieved a 12.34% reduction in wound surface area. Furthermore, the data suggested that patients treated with allo-hBM-MSCDs and allo-hBM-MSCs reached 50% of wound closure after approximately 14 days; in contrast, the patient treated with conventional therapy achieved the same extent of wound closure after approximately 21 days (Fig. 1B). Similarly, changes in wound size were noticed earlier in the allo-hBM-MSCD and allo-hBM-MSC treated patients compared to the conventional treatment. In particular, after 1 day of treatment with either allo-hBM-MSCDs or allo-hBM-MSCs, wounds showed changes in area, volume as well as max depth dimension; conversely, the patient treated with conventional therapy showed changes in these descriptors at 7, 28 and 3 days, respectively (Supplementary Fig. A3). As shown in Table 4, at week 4, allo-hBM-MSCD and allo-hBM-MSC treated patients presented higher decreases in values for wound area, volume and max depth (69.34% vs 90.5%, 93.75% vs 100% and 67.65 vs 96.43%, respectively), compared to the patient treated with the conventional approach (59.68%, 0% and 66.67%, respectively). At week 6, allo-hBM-MSCD and allo-hBM-MSC treated patients showed a total reduction in wound area, volume and max depth, while the patient treated with the conventional approach only achieved a decrease of 76.25%, 16.66% and 66.67%, respectively.

Table 3 Wound baseline characteristics.

Case Number	Wound type	Wound margins	Surrounding skin	Exudate		Signs of critical colonization or infection	Tissue type	Tissue involved in the would	Wound pain
				Volume	Type				
1	Oral	Punched out	Dry/scaly/hyperkeratosis	None	-	None	Granulation (100 %)	Epidermis, Dermis, subcutaneoud tissue	Absent
2	Irregular	Punched out	Dry/scaly/hyperkeratosis	None	-	None	Granulation (100 %)	Epidermis, Dermis, subcutaneoud tissue	Intermittent
3	Irregular	Punched out	Dry/scaly/hyperkeratosis	Moderate	Serous	None	Granulation (100 %)	Epidermis, Dermis, subcutaneoud tissue	Absent

On the other hand, a more rapid macroscopic change on tissue, such as greater re-epithelialization, was observed in patients treated with allo-hBM-MSCDs and allo-hBM-MSCs at day 3, compared to the patient treated with conventional approach (Fig. 2). Specifically, data showed a decrease of approximately 20% and 30% on granulation tissue in patients treated with allo-hBM-MSCDs and allo-hBM-MSCs, respectively; in contrast, a reduction on this tissue was noticed until day 7 for the patient treated with conventional approach (Fig. 2A). Similarly, allo-hBM-MSCD and allo-hBM-MSC treatments stimulated an earlier formation of epithelial tissue, relative to the conventional treatment (day 3 vs day 7, respectively) (Fig. 2B). In fact, replacement of granulation tissue by epithelial tissue was achieved after 35 and 42 days in patients treated with allo-hBM-MSCs or acellular derivatives, respectively; nevertheless, the patient treated with conventional approach still presented granulation tissue at this time. That said, the presence of allo-hBM-MSCDs and allo-hBM-MSCs supported an accelerated and complete wound healing process relative to the conventional treatment.

Discussion

Mesenchymal stem cells have been currently explored as an attractive and harmless therapeutic agent to treat skin lesions. Nevertheless, recent studies have suggested that the tropic factors that MSCs produce are responsible to orchestrate different cellular processes that lead to its restorative effect. Herein, we evaluated the effect of allo-hBM-MSCDs in grade 2 DFUs as a novel healing-guided approach. To our knowledge, this is the first proof of concept that assessed the progress of human DFU healing by the presence of allo-hBM-MSCDs. In particular, we demonstrated that injecting allo-hBM-MSCDs accelerated wound closure in a similar grade as allo-hBM-MSCs, resulting in improved healing, relative to conventional treatment.

Notably, the role of MSCs on wound healing is primarily reflected on both the repair and replacement of cellular substrates and the enhanced wound closure rates, tensile strength and angiogenesis (5, 6). Our findings are in agreement with these facts, since increased wound closure kinetics were evident at day 3 after allo-hBM-MSC and allo-hBM-MSCD administration; conversely, this effect was less marked with conventional treatment and occurred until day 7. These observations suggest that the effect of allo-hBM-MSCs and allo-hBM-MSCDs might ensue in response to, at least, two key mechanisms:

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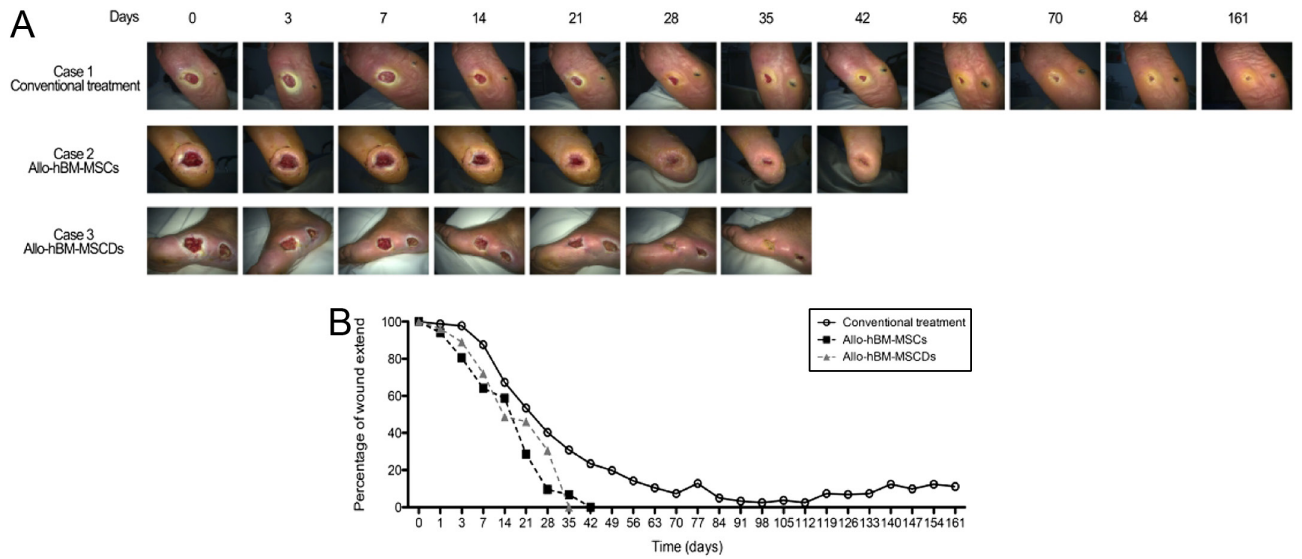

Figure 1
Evolution of wound healing kinetics after intradermal administration of allo-hBM-MSCDs in a patient with grade 2 DFU. (A) Macroscopic analysis of the chronic wound healing progress before and after intradermal administration of 1 mL vehicle, 1×10^6 allo-hBM-MSCs or 1 mL allo-hBM-MSCDs. (B) Percentage of wound extent is represented as the percentage of the surface area of the actual wound related to the surface area of the original wound over time. allo-hBM-MSCDs, allogenic human bone marrow mesenchymal stem cells derivatives; allo-hBM-MSCs, allogenic human bone marrow mesenchymal stem cells; DFU, diabetic foot ulcer.

(1.) MSC transdifferentiation into epithelial cells (8) and/or (2.) the secretion of bioactive soluble factors including growth factors, cytokines and other specific proteins (9). In this context, most studies agree on the fact that, *in vivo*, although MSC migration can be induced by chemotactic signals to sites of injury, only a small percentage of the engrafted MSCs become incorporated and survive within the damaged tissue. Also, a number of studies revealed that the implantation time of MSCs is usually too short to have an effective impact, while others indicated that transplanted MSCs do not necessarily have to be in close proximity to the damaged tissue in order to promote wound repair and functional recovery. Accordingly, various investigations using animal models (including ours) discern that paracrine factors appear to be the leading MSC-therapeutic element entailed in repair of skin lesions (5, 6).

The suited progress of the wound healing process depends on a regulated secretion of growth factors, cytokines and chemokines that are involved in a complex interplay of signals that coordinate cellular processes (10). Non-healing wounds have been associated with the overproduction or insufficient presence of growth factors; for instance, EGF, IGF-1, FGF-2, PDGF-BB, VEGF, Ang-1, SDF-1, KGF, MMP-9, or cytokines such as TGF-β, IL-1, IL-6, IL-8, and TNF-alpha (10). These biomolecules could contribute toward wound repair and skin regeneration by suppressing inflammation, proming angiogenesis and stimulating skin stem cell proliferation and differentiation into new keratinocytes (10). In order to examine the presence of bioactive molecules implicated in the wound healing process, we made a proteomic analysis of the allo-hBM-MSCDs and detected a set of key molecules including IGF-1, EGF, CoL-1, TNF-alpha, MMP-9, PGE2, among others (data not shown). These factors have been listed to possibly promote endothelial cell proliferation and neovascularization as well as restore the dermal architecture and suppress the immune cell response

Table 4 Changes in wound size (area, volume and max depth) at study week 4 and 6.

Week	Wound area			Wound volume			Max depth		
	Case 1	Case 2	Case 3	Case 1	Case 2	Case 3	Case 1	Case 2	Case 3
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	59.68	90.50	69.34	0.00	100	93.75	66.67	96.43	67.65
6	76.25	100	100	16.66	100	100	66.67	100	100

Data are presented as reduction percentage (%).

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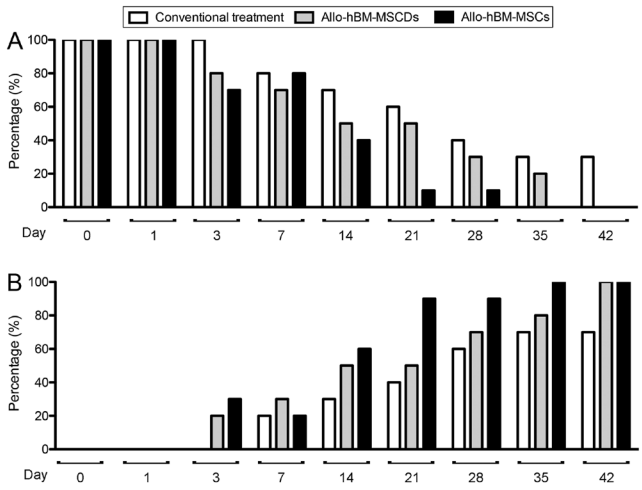

Figure 2
Accelerated macroscopic changes on tissue appearance after intradermal administration of allo-hBM-MSCDs in a patient with DFUs. (A) A more rapid reduction of granulation tissue and (B) a more rapid re-epithelialization were observed in allo-hBM-MSCD and allo-hBM-MSC treated patients, compared to the patient treated with the conventional approach. allo-hBM-MSCDs, allogenic human bone marrow mesenchymal stem cells derivatives; allo-hBM-MSCs, allogenic human bone marrow mesenchymal stem cells; DFU, diabetic foot ulcer.

(10). Moreover, these biomolecules have been proposed as crucial regulators of wound re-epithelialization, supported in our results. Indeed, a more rapid macroscopic replacement on tissue (granulation tissue by epithelial tissue) was noticed in patients treated with allo-hBM-MSCDs and allo-hBM-MSCs relative to the patient treated with conventional approach. These data suggest that MSC treatments induce a more mature stage in granulation tissue that may lead to an accelerated re-epithelialization and improve the wound healing process (11).

Despite these findings, several advantages of using MSCD vs MSC treatment merit comment. MSCDs may become a more potential and available tool for treating DFUs compared to MSCs since they can be stored and administered immediately instead of waiting for cell isolation and expansion. Also, their production at a large scale is both less complex and expensive and their biological activity has been assessed after 17 months of storage opposed to the deleterious effects of cryopreserved MSCs. Regarding the production process, MSCD lots may have higher reproducibility among them due to lower methodological variations against the laboratory-dependent expansion protocols associated to MSC culture. In addition, MSCD administration can be performed by non-medical professional vs presence physician under biosafety considerations. Although MSCDs appear to be a promising option to treat DFUs; phase I/II clinical trials are required to demonstrate their safety and effectiveness. Our cumulative results suggest that combining intradermal administration of allo-hBM-MSCDs with a wound dressing in patients with grade 2 DFU enhances the wound healing process in a similar way than it was observed for patients treated with allo-hBM-MSCs and a wound dressing. Thus, our case report is clinically relevant, as it highlights the possible use of allo-hBM-MSCDs as a novel therapeutic approach to treat DFUs, which could be part of the comprehensive management of DFUs.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EDM-19-0164.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent
Written informed consent was obtained from both bone marrow donors and study participants.

Author c.ontribution statement
M L A and L C M conceived and planned the clinical study design, M V carried out patient wound healing, M L A, J P and M V carried out the follow-up of the patients, S B and M L A wrote the manuscript and V S, C L S and L C M provided critical feedback to the final version of the manuscript.

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