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Therapeutic use of autologous hematopoietic bone marrow transplant combined with concentrated platelet in infected surgical wounds: single-center prospective study

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Abstract

The healing wound is the consecutive process of maintaining tissue integrity including inflammatory, proliferative, and remodeling processes. Stem cells can self-renew, and also to differentiate into all cell types, and are involved in physiological regeneration, use of mesenchymal stem cells deriving from the bone marrow with PRP has potentiated the healing process. We conducted a single-arm pilot prospective analytical study was performed on 15 patients whose mean age was 31 years (range: 29–51 years), with refractory abdominal wounds at a private hospital in Iraq from March 30, 2022, to March 2023. Ten days after the stem cell transplant the re-enforcement with PRP subcutaneous injection around wounds. Small papules of granulation tissue appeared over the wound, and tissue granulation increased from 80% (30.26–80.00%) to 100% (81–100%). At the same time, the wound size was reduced by 60% (52.10–78.23%). The follow-up period was 7–24 months, during which no recurrence of the wound was found. In conclusion, the current study is the first to characterize the safety and efficacy of bone marrow-derived stem cells in combination with PRP in the treatment of refractory abdominal wound infection. All patients achieved a 100% healing rate in a short time, and no treatment-related adverse reactions occurred during the treatment.

Keywords: Bone marrow, Stem cells, PRP, Wound infections

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Introduction

The skin has several layers along with different compounds and roles that work together to support internal organs and serve various biological roles. It has three main layers, the epidermis, the dermis, and the subcutaneous layer. Generally, skin wound healing, triggered by tissue injury, includes four stages: hemostasis, inflammation, proliferation, and maturation [1]. MSCs can assist in all stages of the wound-healing process. The use of MSCs for the treatment of skin can improve the regeneration of skin and reduce scarring. MSCs exert their functions through migration into the skin damage site, suppressing inflammation, and increasing the growth and differentiation ability of fibroblasts, epidermal cells, and endothelial cells [2].

MSCs chiefly found in the bone marrow (BM) possess the ability of self-renewal and also display multilineage differentiation. They were obtained from various tissues and organs including BM, adipose tissue, Wharton's jelly, peripheral blood, umbilical cord, placenta, amniotic fluid, and dental pulp [3]. MSCs can express a wide range of surface markers and cytokine profiles according to the origin of isolation [5]. Nevertheless, the common characterization markers of MSCs are CD73, CD105, CD90 and lacking expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR [4]. During the last decades, MSCs have shown various biological roles such as multilineage differentiation, immunomodulation, angiogenesis, anti-apoptotic and anti-fibrotic activity, chemo-attraction, and tissue repair development [6]. The MSCs have broad properties that make them a suitable source for cell therapy, such as stemness potency, and easy isolation from different sources, they can be rapidly expanded in a large scale for clinical use, and have less ethical issues as compared to ESCs, unlike iPSCs, MSCs transport a lower risk of teratoma formation, and they are beneficial for a wide scale of therapeutic applications due to their capability to migrate to injured tissue through chemo-attraction [7]. In addition, MSCs can release a variety of bioactive components including proteins, growth factors chemokines, microRNAs (miRNAs), and cytokines which can suggest their acceptable application [8].

Patients and Methods

We conducted a single-arm pilot prospective analytical study was performed on 15 patients whose mean age was 31 years (range: 29–51 years), with refractory abdominal wounds at a private hospital in Iraq from March 30, 2022, to March 2023. This study was approved by the local Ethics Committee and the study adhered to the Declaration of Helsinki.

Preparation of autologous bone marrow aspiration

Hematopoietic bone marrow was harvested from the posterior iliac crest of each patient. A small incision was made in the region of the posterior iliac crest, and a bone marrow aspiration needle was inserted between the posterior crest cortical walls. A total of 150–200 mL of bone marrow aspirate was obtained, which was transferred to a sterile pouch containing anticoagulants such as acid-citrate-dextrose and sodium citrate. The aspirated bone marrow specimen was washed under sterile conditions through the class II laminar flow and filtered to eliminate lipids and clot particles. Through a cold centrifuge of 1720 g for 10 minutes, remove the above layer and a buffy coat and move to another sterile plastic tube for further concentration. The supernatant containing stem cells was then removed and collected in other sterile centrifuge tubes for subsequent use as we published [9].

platelet-rich plasma

To prepare allogeneic PRP, platelets were obtained from patients using apheresis (Jiangsu Province Blood Center, Nanjing, China). Each 250–300 mL donor unit had at least 2.5×1011 platelets suspended in plasma. containing approx. 30 mL of anticoagulant citrate dextrose solution (formula A (ACD-A)) Each unit was subdivided into 10–20-milliliter portions using a 4-bag system (Fresenius Kabi, Guangzhou, China). Next, all aliquots of PRP were frozen at −80°C22,23 until further use. 0.5 mL of them was collected within 30 min and a platelet count was taken for each unit of PRP before

cryopreservation, using the XE2100™ automatic hematology analyzer (Sysmex Corporation, Hyogo, Japan). Each unit of PRP was tested for the presence of aerobic and anaerobic bacteria using Plus Aerobic/F and Lytic/10 Anaerobic/F (9184994 and 0301023; BD Biosciences, Franklin Lakes, USA), respectively. Calcium gluconate (10%) was added to each PRP sample at a ratio of 1:4 in a 37°C water bath. The PG formed within a few minutes. To isolate platelet growth factors (GFs), PG was ultracentrifuged at 10,000 × g for 15 min to eliminate fibrous protein and platelet debris, and the GF concentration in the supernatant was measured. The following 3 GFs were evaluated: transforming growth factor beta 1 (TGF-β1), platelet-derived growth factor BB (PDGF-BB) and vascular endothelial growth factor (VEGF). To change the latent form into an immune-reactive form, the content of TGF-β1 was tested after acidic activation and neutralization of the sample. The GF concentrations were measured using Human VEGF enzyme-linked immunosorbent assay (ELISA) Kit (A18310135; Multi Sciences, Hangzhou, China), Human/Mouse/Rat TGF-β1 ELISA Kit (A98110121; Multi Sciences), and Human/Rat PDGF-BB ELISA Kit (A913700823; Multi Sciences), and were tested using Hamilton Microlab FAME (M8; Hamilton Corporation, Bonaduz, Switzerland). All of the above tests were performed before PG treatment.

Treatment procedure

To minimize the residual infection risk, the refractory wounds were first debrided to remove the necrotic and infected tissues. After that, the wound was rewashed with normal saline and dried with sterile gauze. Wound volume was calculated as lesion area × depth. Based on the wound size, the appropriate volume of multi-injection S.C. around the wound mixture of stem cells and PRP. The dressing was changed every day and wound healing was observed. During the therapy, the patient's wounds were not treated with therapeutic agents containing basic fibroblast GFs or with negative pressure wound therapy. Wounds were photographed before each treatment and during the follow-up visit using a digital camera, and examined for abnormalities such as bleeding, exudation, infection, and poor wound healing. These observational indexes were used to evaluate the therapy. Several parameters were selected to evaluate the efficacy of therapy, including the presence of infection, granulation growth in wounds, wound healing rate, and healing time. If the patient had a skin graft after therapy, graft survival, and graft edema were assessed after the transplantation.

Statistical analysis

In this cross-sectional, descriptive study, the mean age and incidence of cytogenetic abnormalities, using the SPSS software package (version 18). Moreover, we performed comparisons in terms of cytogenetic subclasses and age groups using the Pearson chi-square test with MED CALC software.

Results

Fifteen patients, having refractory abdominal wounds not healed after extensive conventional wound management, were included, and treated with S.C. administration of stem cells derived from bone marrow and PRP. Their mean age was 37 years (range: 31–55 years. The characteristics of patients are presented in Table 1.

Table 1.

Characteristics of the study population (n = 15)

Parameter		Number of patients (n)
Age	29–33	3
	34–38	6
	39–43	3
	44–48	2
	49-53	1
Sex	Male	6
	Female	9
Type of abdominal operation	Abdominal plastic	5
	Scope abdominal surgery	2
	Open abdominal surgery	8

Ten days after the stem cell transplant re-enforcement with PRP treatment, small papules of granulation tissue appeared over the wound, and tissue granulation increased from 80% (30.26–80.00%) to 100% (81–100%). At the same time, the wound size was reduced by 60% (52.10–78.23%). The follow-up period was 7–24 months, during which no recurrence of the wound was found.

Discussion

The current study is the first to characterize the safety and efficacy of bone marrow-derived stem cells in combination with PRP in the treatment of refractory abdominal wound infection. All patients achieved a 100% healing rate in a short time, and no treatment-related adverse reactions occurred during the treatment. Refractory wounds are one of the outcomes post-operations and are challenging to treat. Conventional treatment for refractory wounds includes control of infection, adequate debridement, avoidance of excessive pressure, revascularization of ischemic tissue, changing the wound environment, and medical management of comorbidities. The first phase of wound healing is the inflammatory phase, which begins at the time of wounding [10]. Activation of the coagulation cascade initiates the release of cytokines and chemokines which stimulate the chemotaxis of neutrophils, followed by macrophages and later lymphocytes, into the wound for debridement [11]. These inflammatory cells, in turn, secrete growth factors and provisional matrix proteins which promote the recruitment of neighboring epidermal and dermal cells to the wound bed [12]. Inflammation controls microbial invasion and clears the wound site of cellular debris; however, prolonged inflammation can result in the formation of scar tissue [13]. On the other hand, an absent or inadequate inflammatory response can give rise to chronic, nonhealing wounds. Once present at sites of injury, MSCs exert immunosuppressive effects [14]. Wounds treated with MSCs have lower numbers of inflammatory cells and proinflammatory cytokines, such as IL-1 and TNFα [15]. Upon exposure to proinflammatory cytokines, including IFN-γ, TNFα, IL-1α, and IL-1β, the immunosuppressive phenotype of MSCs becomes activated, and they begin to express chemokines and inducible nitric oxide synthase (iNOS), which suppress T cell responsiveness to inflammation. In response to MSC activity, T cells secrete less IFN-γ and more IL-4, and the number of regulatory T cells increases [97–99]. MSCs also regulate the proliferation, differentiation, and function of B cells [100] and natural killer cells [16], causing natural

killer cells to secrete less IFN-γ. Some studies suggest MSCs may differentiate into a variety of skin cells in the healing wounds following transplantation. *In vivo* studies tracing fluorescently labeled BMSCs injected intravenously or topically applied to the wounded skin of mice report the presence of fluorescent cells upon healing that are positive for markers of dermal fibroblasts, endothelial cells, pericytes [17], and epidermal keratinocytes. Several studies have demonstrated that systemically injected MSCs migrate to various sites of injury in the host [18]. The wound's local environment influences the homing and behavior of the MSCs [19-23]. These cells respond favorably to the local inflammatory and hypoxic conditions of the wound environment, and they support important wound healing events such as matrix deposition and blood vessel formation by stimulating the increased rate of proliferation, differentiation, and growth factor production (such as vascular endothelial growth factor [VEGF] and fibroblast growth factor [FGF]) [24]. Recent evidence indicates that VEGF not only supports neovascularization but also enhances keratinocyte proliferation, suggesting a further paracrine role for MSCs in wound re-epithelialization [25-30]. Other studies demonstrate that MSCs and their secreted factors can inhibit T-lymphocyte activation and proliferation [31]. MSCs secrete prostaglandin E2, which downregulates inflammatory cytokines produced by macrophages.

There are encouraging results from human studies using multipotent stem cells as therapeutic agents for tissue repair. Application of autologous bone marrow cells results in complete wound closure with dermal rebuilding and decreased scar in chronic nonhealing wounds that were refractory to conventional therapy.

Conclusion

The present study is the first prospective, randomized, and controlled trial evaluating the efficacy of autologous bone marrow combined with PRP in wound infections for abdominal surgery, and it has demonstrated that BMT and PRP have positive effects on wound healing and pain reduction in those patients undergoing abdominal surgery. The MSCs promote healing in all phases of wound repair that migrate to sites of cutaneous injury and, primarily through paracrine signaling, suppress inflammation and stimulate the proliferation and differentiation of resident progenitor cells including fibroblasts, endothelial cells, and epidermal cells.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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References

1. Schultz GS, Chin GA, Moldawer L, Diegelmann RF. Principles of Wound Healing. Diabet. Foot Probl. 2011;77:395–402.
2. Swart JF, et al. Haematopoietic stem cell transplantation for autoimmune diseases. Nat Rev Rheumatol. 2017;13(4):244–56.
3. Abbaszadeh H, et al. Regenerative potential of Wharton's jelly-derived mesenchymal stem cells: a new horizon of stem cell therapy. J Cell Physiol. 2020;235(12):9230–40.
4. Chimutengwende-Gordon M, Khan WS. Advances in the use of stem cells and tissue engineering applications in bone repair. Curr Stem Cell Res Ther. 2012;7(2):122–6.
5. Yu Y, et al. Human embryonic stem cell-derived cardiomyocyte therapy in mouse permanent ischemia and ischemia-reperfusion models. Stem Cell Res Ther. 2019;10(1):167.
6. Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. Science. 2000;290:328–330.
7. Saldaña L, et al. Immunoregulatory potential of mesenchymal stem cells following activation by macrophage-derived soluble factors. Stem Cell Res Ther. 2019;10(1):58.
8. hi R, et al. Localization of human adipose-derived stem cells and their effect in repair of diabetic foot ulcers in rats. Stem Cell Res Ther. 2016;7(1):155.
9. Marfia G, et al. Mesenchymal stem cells: potential for therapy and treatment of chronic non-healing skin wounds. Organogenesis. 2015;11(4):183–206.
10. Hu MS, et al. Mesenchymal Stromal Cells and Cutaneous Wound Healing: A Comprehensive Review of the Background, Role, and Therapeutic Potential. Stem Cells Int. 2018;2018:6901983.
11. Abdollahi H, Harris LJ, Zhang P, et al. The role of hypoxia in stem cell differentiation and therapeutics. J. Surg. Res. 2011;165:112–117.
12. Hsu YC, Li L, Fuchs E. Emerging interactions between skin stem cells and their niches. Nat Med. 2014;20(8):847–56.
13. Brennan MÁ, Layrolle P, Mooney DJ. Biomaterials functionalized with MSC secreted extracellular vesicles and soluble factors for tissue regeneration. Adv. Funct. Mater. 2020;30:1909125.
14. Branski LK, Gauglitz GG, Herndon DN, Jeschke MG. A review of gene and stem cell therapy in cutaneous wound healing. Burns 2009; 35:171-80.
15. Atiyeh BS, Ioannovich J, Al-Amm CA, El-Musa KA. Management of acute and chronic open wounds: the importance of moist environment in optimal wound healing. Curr Pharm Biotechnol. 2002;3:179-95.
16. Chance TC, Rathbone CR, Kamucheka RM, et al. The effects of cell type and culture condition on the procoagulant activity of human mesenchymal stromal cell-derived extracellular vesicles. J. Trauma Acute Care Surg. 2019;87:S74–S82.
17. Hernigou P, et al. Subchondral bone or intra-articular injection of bone marrow concentrate mesenchymal stem cells in bilateral knee osteoarthritis: what better postpone knee arthroplasty at fifteen years? A randomized study. Int Orthop. 2021; 45(2):391–9.

18. Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. Lancet 2005; 366:1719-24.
19. Maxson S, Lopez EA, Yoo D, et al. Concise review: role of mesenchymal stem cells in wound repair. Stem Cells Transl Med. 2012;1:142-9.
20. Dabral S, Muecke C, Valasarajan C, et al. A RASSF1A-HIF1α loop drives Warburg effect in cancer and pulmonary hypertension. Nat. Commun. 2019;10:2130.
21. Chahal J, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. Stem Cells Transl Med. 2019;8(8):746–57.
22. Gibran NS, Heimbach DM. Current status of burn wound pathophysiology. Clin Plast Surg. 2000;27:11-22.
23. Silva I, Almeida J, Vasconcelos C. A PRISMA-driven systematic review for predictive risk factors of digital ulcers in systemic sclerosis patients. Autoimmun Rev. 2015;14:140-52.
24. Eberly AR, Elvert JL, Schuetz AN. Best practices for the pre-analytic phase of anaerobic bacteriology. Clin. Microbiol. Newsl. 2022;44, 63–71.
25. Ko SH. Nauta A. Wong V. Glotzbach J. Gurtner GC. Longaker MT. The role of stem cells in cutaneous wound healing: what do we really know? Plast Reconstruct Surg. 2011;127(Suppl 1):10S.
26. Stappenbeck TS. Miyoshi H. The role of stromal stem cells in tissue regeneration and wound repair. Science. 2009;324:1666.
27. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284:143.
28. Lee EY, Xia Y, Kim WS, et al. Hypoxia-enhanced wound-healing function of adipose-derived stem cells: increase in stem cell proliferation and up-regulation of VEGF and bFGF. Wound Repair Regene. 2009;17:540.
29. Maggini J. Mirkin G. Bognanni I, et al. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One. 2010;5:e9252.
30. Mohyeldin A. Garzon-Muvdi T. Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell. 2010;7:150.
31. Falanga V, Iwamoto S, Chartier M, et al. Autologous bone marrow-derived cultured mesenchymal stem cells delivered in a fibrin spray accelerate healing in murine and human cutaneous wounds. Tissue Eng. 2007;13:1299.

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