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An evaluation of the clinical effectiveness of autologous bone marrow cells as a supplement to traditional therapy for wound healing in canine

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Abstract

A wound is defined as a breach in the integrity of tissue. Wound healing is an essential requirement of all surgical manipulation, and has the focus of various treatment strategies aimed at enhancing this process. The present retrospective study was carried out with an objective to evaluate the comparative efficacy of local transplantation of autologous whole bone marrow (WBM) in conjunction with conventional therapy versus conventional therapy alone in promoting wound healing in dogs. A total of 14 clinical cases of dogs were randomly selected, irrespective of breed, age, sex, and reproductive status, and divided into two equal groups – Group A and Group B. The dogs in Group A received only conventional wound management, while Group B dogs were treated with local transplantation of autologous WBM along with conventional therapy. The rate of wound healing was accessed on the basis of clinical parameters including appetite, level of activity, visual analogue score (evaluating pain, swelling, and exudation), total healing (on days 0, 3, 7, and 10), and the total number of days required for complete healing/ suture removal. The statistical analysis of the collected data was performed by software SPSS 17, using two-way analysis of variance (ANNOVA). On the basis of clinical findings, it was concluded that addition of local transplant of autologous WBM to conventional wound management therapy significantly increased the quality and rate of wound healing in dogs compared to conventional therapy alone.

1. Introduction

Wound can be defined as breach in the continuity of skin or underlying soft tissues. The healing of such wounds involves a tightly regulated process of three essential phases: inflammation, angiogenesis, and tissue remodelling (Sim et al. 2022). This process involves the exchange of cytokines and growth factors, which promotes cell migration to the injured area. The inflammatory phase, being the first phase of wound healing, starts within 6–8 hours of injury. In order to prepare the tissue for the proliferative phase, platelets move to the tissue and produce chemo-attractive cytokines that facilitate the recruitment of immune cells. Macrophages arrive and perform phagocytosis to clear debris and pathogens (Sim et al. 2022). Five to seven days following injury, the proliferative phase begins, triggered by cytokines secreted by macrophages such platelet-derived growth factor (PDGF), transforming growth factors (TGF-α/β), and fibroblast growth factor (FGF) (Golebiewska and Poole 2015). Granulation tissue is formed during this stage as a result of extracellular matrix deposition and fibroblast proliferation. Concurrently, angiogenesis takes place during this stage, facilitating leukocyte movement and supplying oxygen and nutrients for the development of granulation tissue.

Tissue remodelling, the last phase, results in mature scar development over a period of months to years of wound contraction and extracellular matrix restructuring. Adequate oxygenation of the tissue, a sufficient quantity of growth factors, nutrients, coordinated cellular interactions, and other factors are necessary for an effective wound-healing process. Systemic conditions like infection, malnutrition, diabetes, or other chronic illnesses can impair these mechanisms, often resulting in delayed wound healing and the development of chronic wounds. Chronic wound care has limited success and treatment choices are restricted, despite optimal local wound care and systemic causes, such as blood glucose levels and tissue oxygenation, are addressed (Werdin et al. 2008). Stem cell therapy has emerged as a promising approach to enhance wound healing. Stem cells have been investigated for their regenerative potential in skin injuries, neurological diseases, and ischemic tissue damage such as peripheral artery disease, chronic diabetic wounds, and venous ulcers (Kim et al. 2009; Nakagami et al. 2005; Kosaric et al.

2019).

Stem cells from diverse sources, such as endothelial progenitor cells (EPCs), dermal stem cells (DSCs), bone marrow-derived mesenchymal stem cells (BM-MSCs), adipose derived stem cells (ASCs), and induced pluripotent stem cells (iPSs) have been employed regenerative medicine. These stem cells promote wound healing through paracrine signaling and the secretion of growth factors, which in turn promote fibroblast proliferation and tissue remodeling (Nakagami et al. 2005). A wide variety of cells make up bone marrow-derived mononuclear stem cells (BMMCs), including mature B and T lymphocytes, monocytes, endothelial progenitor cells, embryonic-like cells, and the cells expressing CD 133, CD 117, and CD 34. BMMCs are a practical and rich source of stem cells, readily obtained from peripheral blood and bone marrow without the need for expansion in vitro (Amato et al. 2016). In this context, the present study was therefore planned with an objective to analyze the clinical data and to compare the clinical efficacy of autologous bone marrow cell (BMC's) therapy, as an adjunct to conventional management, in promoting wound healing in dogs.

2. Materials and methods

The present study conducted on a total of 14 dogs presented to the Veterinary Clinical Complex (VCC) for treatment of fresh traumatic cutaneous wounds, within 2-3 hours of injury. The dogs were randomly divided into two groups of seven animals each - Group A and Group B – irrespective of their breed, age, sex, and reproductive status. The assessment of rate of wound healing was done on the basis of clinical parameters, such as level of activity, appetite, degree of inflammation (pain at the site, swelling, and exudation), presence of infection, wound dehiscence (if any), and the number of days required for complete clinical healing/ suture removal. The observations were recorded at specific intervals – day 0, day 3, day 7, and day 10 during the course of treatment. Each clinical parameter, including pain, degree of swelling, degree of exudation, along with the total inflammatory and healing scores, was evaluated using a scoring system as described by Gangwar (2002).

3. Results and Discussion

Pain was significantly present in both groups on day 0. On day 3, the mean pain score has reduced to 2.67 ± 0.21 in group A and 2.00 ± 0.00 in group B. On day 7, a significant differences were noted, with further reduction in mean pain scores of Group B (1.00 ± 0.36) compared to 2.67 ± 0.21 in group A. On day 10, pain was clinically negligible in both groups (Table 1). The early reduction of pain in the treatment group might be attributed to a faster healing process, probably due to the growth factors present in whole bone marrow (Rodriguez-Menocal et al. 2012).

Swelling was significantly present in both groups on day 0. On day 3, the mean swelling scores were 2.17 ± 0.17 in group B and 2.50 ± 0.22 in group A. By day 7, significant differences were noticed in mean swelling scores with mean of 1.50 ± 0.43 in group A and 1.00 ± 0.36 in group B. On day 10 swelling had resolved completely in group B (0.00 ± 0.00), but a residual swelling (0.17 ± 0.17) was still observed in Group A (Table 1).

On day 0, there was a distinct exudation in all the animals of both groups. On day 3, the mean exudation score was 2.50 ± 0.22 in group A and lower score of 2.00 ± 0.00 was observed in Group B. On day 7, exudation was further reduced to 1.50 ± 0.34 in group A and 0.83 ± 0.31 in group B. On day 10, the exudation got resolved completely in both groups (Table 1).

The mean **Overall Inflammatory** score was obtained by summation of individual scores of pain, swelling, and exudation. On day 0, marked inflammation was present in all cases. On day 3, the inflammation reduced 6.17±0.17 in group B and 7.67±0.33 in group A. On day 7, the inflammatory score in group B had significantly reduced (2.83±1.010 compared to group A (4.33±1.05). On day 10, the inflammation got resolved in Group A, whereas slight inflammatory signs were still present in group A (0.50± 0.34) (Table 1).

Initial short haemostasis phase is generally followed by the inflammatory phase of wound healing with characteristic signs of inflammation which includes erythema, swelling, warmth, and pain.

Table 2. Mean (±SE) Scores of different parameters at different time intervals between the groups

Parameters	Group	Day 0	Day 3	Day 7	Day 10
Pain score	A	3.00^{aC}±0.00	2.67^{bC}±0.21	1.33^{aB}±0.33	0.33^{aA}±0.21
	B	3.00^{aD} ±0.00	2.00^{aC}±0.00	1.00^{aB}±0.36	0.00^{aA}±0.00
Swelling score	A	2.67^{aC}±0.33	2.50^{aC}±0.22	1.50^{aB}±0.43	0.17^{aA}±0.17
	B	2.50^{aC}±0.22	2.17^{aC}±0.17	1.00^{aB}±0.36	0.00^{aA}±0.00
Exudation score	A	2.67^{aC}±0.33	2.50^{aC}±0.22	1.50^{aB}±0.34	0.00^{aA}±0.00
	B	2.17^{aC}±0.31	2.00^{aC}±0.00	0.83^{aB}±0.31	0.00^{aA}±0.00
Overall inflammatory score	A	8.33^{aC}±0.67	7.67^{aC}±0.33	4.33^{aB}±1.05	0.50^{aA}±0.34
	B	7.67^{aC}±0.42	6.17^{bC}±0.17	2.83^{aB}±1.01	0.00^{aA}±0.00
Total healing score	A	0.00^{aA}±0.00	1.00^{aB}±0.00	2.17^{aC}±0.17	2.67^{aD}±0.21
	B	0.00^{aA}±0.00	1.17^{aB}±0.17	2.33^{aC}±0.21	3.00^{aD}±0.00

Means bearing different superscript in small alphabets show significant differences between groups at same time intervals, while means bearing different superscript in capital alphabets show significant differences within the groups at different time intervals ($p \leq 0.05$)

The inflammatory phase increases vascular permeability, which aids in migration of neutrophils and monocytes into the surrounding tissue. The neutrophils engulf debris and microorganisms, providing the first line of defense against infection. Neutrophil migration ceases after first few days of injury, if the wound is not contaminated. If this acute inflammatory phase persists due to wound hypoxia, infection, nutritional deficiencies, medication use, or other factors related to the patient's immune response, it can impede wound healing (Stadelmann et al. 1998; Mackay and Miller 2003).

The mean **total healing score** was zero at day 0. On day 3, it increased to 1.17±0.17 in group B and 1.00±0.00 in Group A. On day 7, healing score progressed to 2.33±0.21 in group B and 2.17±0.17 in group A. By day 10, complete healing was observed in Group B with a mean score of 3.00±0.00, whereas Group A still had incomplete healing with a mean score of 2.67±0.21 (Table 1). Complete healing was seen in Group B probably due to the inherent regenerative potential whole bone marrow cells to rebuild the dermis by differentiating into various cell types such as fibroblasts, cartilage, and muscle (McFarlin et al. 2006). For complete healing or suture removal, Group A required an average of 9.33±0.76 days, which was slightly higher than group B with an average of 8.17±0.65 days (Fig. 1).

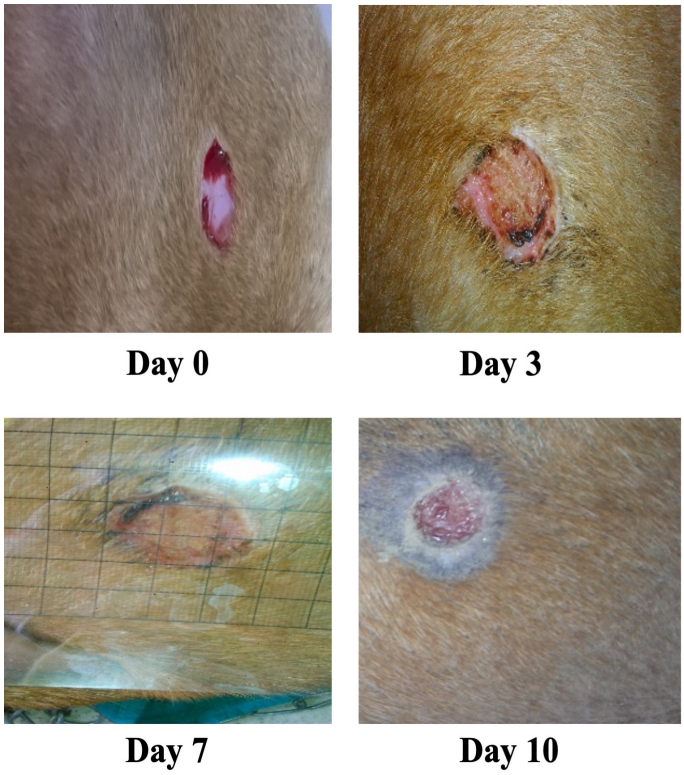

Fig. 1. *Wound healing progress from day 0 to day 10*

4. Conclusions

In the present study, augmentation of local transplantation of fresh autologous whole bone marrow to the conventional treatment (regular antiseptic dressing, systemic administration of ceftriaxone @10 mg/ Kg body weight and meloxicam @ 0.3 mg/ Kg body weight), enhances wound healing in dogs. Based on the retrospective clinical observations, it can be concluded that autologous whole bone marrow transplant, as an adjunctive therapy to conventional treatment, significantly accelerates the wound healing process. Therefore, the use of whole bone marrow offers a promising and clinically viable therapeutic modality for clinical cases of traumatic or surgical cutaneous wounds in dogs. Furthermore, the study opens avenues for future research on the combined use of whole bone marrow cells and platelet-rich plasma. While whole bone marrow offers a reservoir of diverse cell populations, including mesenchymal stem cells, platelet-rich plasma is a rich source of growth factors that further support wound healing.

Declarations

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