TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Case Report

Turk J Vet Anim Sci
(2015) 39: 115-119

doi:10.3906/vet-1404-95

Cell-based therapy and rehabilitation with prosthetic limbs in a dog

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Received: 23.04.2014 • **Accepted:** 11.07.2014 • **Published Online:** 12.01.2015 • **Printed:** 09.02.2015

Abstract: A 5-year-old male dog was presented with the complaint of a chronic nonhealing wound on both hind legs. The wound occurred during a serious train accident that caused loss of both hind limbs below the hock joint. Since then, the wound did not respond to any standard treatment for up to 4 months. Again it was dressed for 15 days, but it did not show any improvement. With the consent of the owner, cell-based therapy was conducted by collecting bone marrow from the proximal anteromedial aspect of the tibia. After the application of bone marrow derived mesenchymal stem cells (BM-MSCs) the time to complete healing was 24 days. Due to pressure at the bony cut stump, the dog was noted to have pain while walking, and walked on his forelegs. An artificial limb was subsequently applied. The dog managed to walk normally after several days of training.

Key words: Chronic nonhealing wound, BM-MSCs, dog, rehabilitation

1. Introduction

Wound healing is a highly complex process. It is a matter of major concern for clinicians (1), and has always been a challenge for veterinarians. In comparison to human wounds, animal wound care is challenging due to the very nature and behavior of habit and habitat, which lead to a greater degree of wound complication and infection (2). Nonhealing or slow-healing ulcers represent a major health burden and drain on resources (3), contributing to substantial disability and morbidity (4,5). Chronic nonhealing wounds continue to pose a challenge not only to the physician, but also to the community and society as a whole.

Optimum healing of a cutaneous wound requires a well-orchestrated integration of complex biological and molecular events of cell migration, proliferation, extracellular matrix (ECM) deposition, angiogenesis, and remodeling (6–9). In the field of regenerative medicine, clinical trials suggest that direct application of autologous bone marrow cultured cells may accelerate the healing of nonhealing chronic ulcers (10,11). Bone marrow (BM) contains nonhematopoietic mesenchymal stem cells (MSCs) capable of differentiating into cells of numerous tissue lineages (12). These cells also possess a distinctive ability to renew themselves by mitotic division and to differentiate into a wide spectrum of cells, as seen during embryonic development (13).

2. Case history

A 5-year-old, male, Himalayan, long-haired breed dog was presented with severely wounded nonhealing hind limbs amputated above the hock region (Figure 1). History revealed that the dog had been injured during transportation by train, and was subsequently treated with several standard therapeutic regimens for 4 months without any favorable result. Based on the characteristics of the wound, regenerative medicine, such as stem cell therapy, was chosen as a therapeutic maneuver. With proper consent from the owner, autologous bone marrow derived from mesenchymal stem cell (BM-MSCs) therapy was planned.

3. Treatment

Under injectable general anesthesia using atropine, xylazine, and ketamine, the proximal anteromedial aspect of tibia was prepared aseptically for bone marrow aspiration. A 1-cm cutaneous skin incision was made at the proposed site, and the bone was drilled through the incision with an electrical orthopedic drill bit of 2 mm diameter. Then 10 mL of BM was aspirated into a sterile syringe primed with EDTA (Himedia, India) at 1 mg/mL. The BM aspiration

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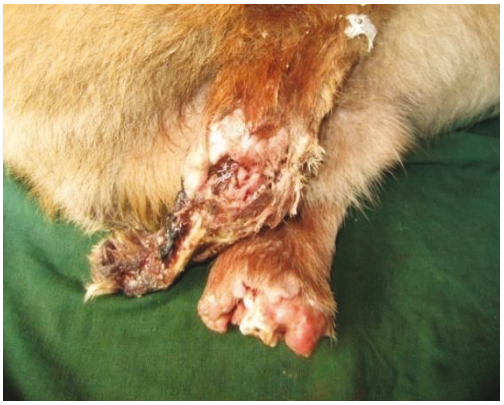

Figure 1. Wound at the time of presentation.

was performed according to the method of Crow and Walshaw (14). The BM-derived nucleated cells (BM-NCs) were collected from aspirate by volume reduction protocol according to the method of Kasten et al. (15). The EDTA-mixed bone marrow was dispatched for culture and growth to the stem cell laboratory of the Central Institute of Freshwater Aquaculture (CIFA), Odisha, India. The conditioned media were prepared for culture with commercially available basic ingredients (Table). The collected bone marrow was layered over Histopaque (Sigma-Aldrich, USA) for the isolation of mononuclear cells, and was centrifuged at 2000 rpm for 20 min. The monocytes in the buffy layer were seeded onto a 0.1% gelatin-coated 6-well plate (Tarson, India), and 100 µL of cells were poured into a 6-well plate containing 2 mL of composed media at 37 °C and 5% CO_2 in the CO_2 incubator (Contherm Scientific Ltd, USA). The medium was changed regularly and cell morphology was examined under a Nikon phase contrast microscope (Figure 2). After complete colony formation and the attainment of confluence, the cultured cells were removed for therapeutic application. The BM-MSCs were injected intradermally and applied topically at the wound margin and at different points in the wound bed (Figure 3). The wound sites were maintained undisturbed for 20 min for proper adherence of the implanted MSCs and were then bandaged with a paraffin wet bandage. During the study, a bacteriological swab was taken to determine the load of microorganisms at the site of the wound. Blood and tissue samples were collected for hematobiochemical, histochemical, and histopathological study. Physical parameters (wound dimension, type and extent of exudates, swelling, and wound contraction) and clinical parameters (rectal temperature, pulse, respiration, and character of visible mucous membrane) were studied. A sequential photographic record was maintained. Pain-free walking distance was also measured during the study.

4. Results and discussion

Ciprofloxacin was administered parenterally along with regular sterile dressing before stem cell application, as per the antibiogram. BM was collected from the proximal anteromedial aspect of the tibia, because of its superficial position with thin covering tissue and ease of collection compared to other sites. The moist wound environment allows optimal healing by hastening debridement and promoting granulation tissue formation and fast epithelialization (16). Hence, after application of BM-MSCs, the wound was bandaged with a paraffin wet bandage to create a better environment for healing and to prevent leakage of MSCs. There was no significant difference in physiological, hematological, or biochemical parameters during the study. Significant improvement in physical parameters was observed during the healing period. Pain-free walking distance gradually increased. Histopathology after therapy showed neovascularization (Figure 4) with appearance of fibroblasts, sebaceous glands, and epithelialization, which supported the progression of the healing process (6–8). Histochemical study showed increased collagen content after stem cell therapy. The collagen content with Sircol assay kit method on days 0, 14, and 16 were 12.76, 24.82, and 28.69 µg/mg, respectively, which supports the findings reported by Ghani et al. (17), Pascoe (18), Curtis (19), and Singh and Singh (20) regarding wound healing. The photographs

Table. Constituents of the composed media.

Constituents of media	Amount
FBS (Fetal Bovine Serum) 10%, Lonza	5 mL
Sodium Pyruvate 0.1%, Himedia	0.5 mL
NEA (Nonessential amino acids) 0.1%, Himedia	0.5 mL
DMEM (Dulbecco-modified Eagle medium) (MP pharmaceuticals)	18.5 mL
Streptomycin (Sigma-Aldrich)	0.5 mL
L 15 (Livosys 15) washing media	25 mL
Total	50 mL

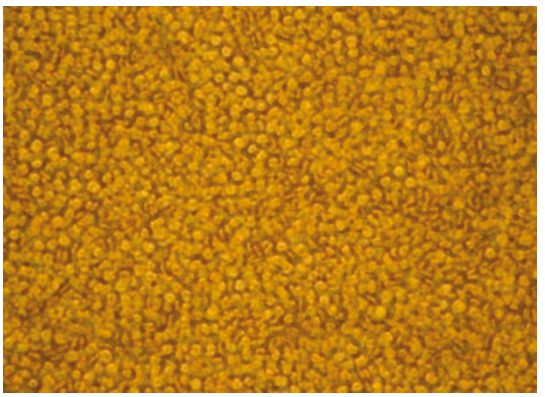

Figure 2. A 40× photomicrograph of cultured BM cells on day 7.

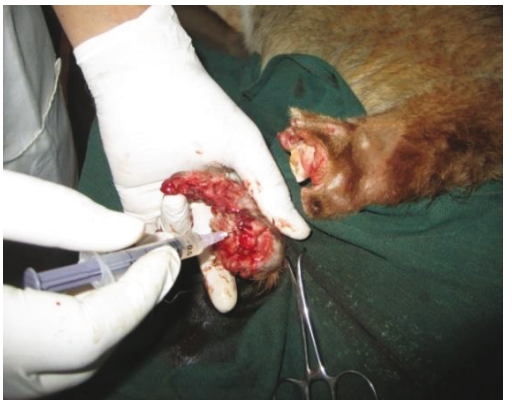

Figure 3. Application of BM-MSCs by intradermal injection.

of the wound were evaluated by 3 surgeons according to Borena et al. (21), and showed complete healing on day 24 (Figure 5). The healing of cutaneous wounds is mediated by the regenerative effects of the transforming growth factor (TGF), fibroblast growth factor (FGF), fibronectin-like peptide, keratinocytes growth factor, epidermal growth

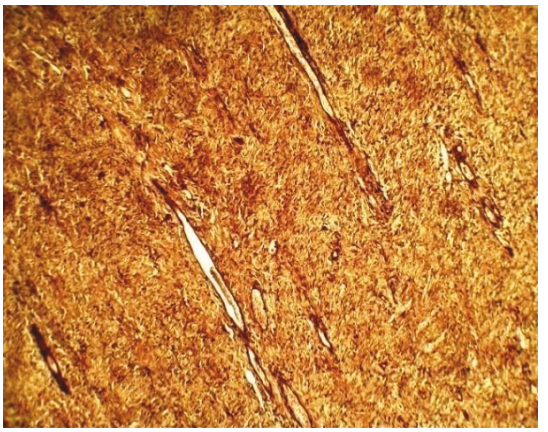

Figure 4. Formation of granulation tissue and abundant neovascularization after application of BM-MSCs.

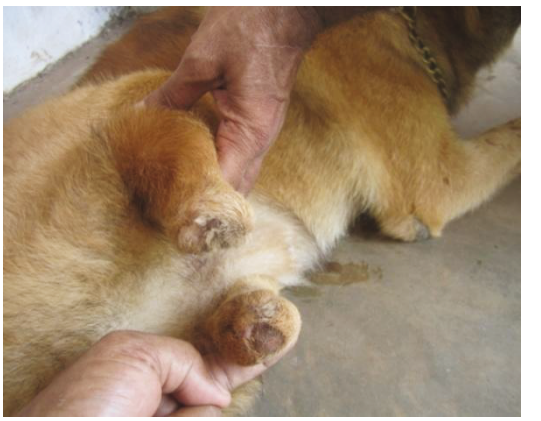

Figure 5. Wound after 24 days of BM-MSCs application.

factor, and growth hormone releasing factors (22). In this case, all these factors were possibly accelerated due to the application of MSCs. The role of MSCs in wound healing is attributed to their ability not only to differentiate but also to produce various angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (23). On day 24 of stem cell therapy, the dog was able to walk moderately following bandaging of the amputated sites (Figure 6). A synthetic shoe was applied as a protective pad to prevent injury to the freshly healed wounds (Figure 7). As a rehabilitation measure, the dog was tried with a wheel cart, but did not accept it for regular use. As a result, it was taken to the National Institute of Rehabilitation Training and Research (NIRTAR), Odisha, India, and a clinical trial was performed with an artificial prosthetic limb (Figure 8). Following 20 days of rehabilitation and training, the dog was able to adopt the prosthetic limbs for regular use. Rehabilitation was followed by 6 months of therapy, and no remarkable untoward effect was noticed. With the application of BM-MSCs and rehabilitation with artificial limbs, the

Figure 6. Application of bandage over amputated end.

Figure 7. Application of shoes.

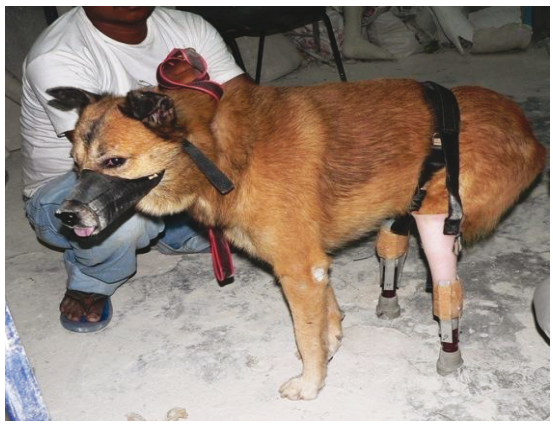

Figure 8. Application of artificial limbs.

injured dog regained mobility and recovered. The stem cell therapy in this study has been shown to be a simple, safe, effective, quick, and nonreactive procedure that can be employed for large-scale application. The artificial limb can also be adopted as a substitute for the wheel cart used for the normal mobility of companion animals.

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