Autologous stem cell therapy to treat chronic ulcer in heifer: A case study

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Abstract

Aim: The study was conducted to reveal the efficacy of Bone marrow derived mesenchymal stem cells (BM-MSCs) based therapy in healing of chronic non-healing and ulcerative wound in bovine species.

Materials and Methods: One 2 years old Jersey heifer affected with chronic ulcerative wound involving full thickness skin and under lying muscle at dorsal side of lumbar region since four months at the time of presentation. Bone marrow was collected from tibia, cultured and grown and after achievement of optimum confluence it was applied at the site. Different parameters of clinical, physiological, haematological, biochemical, histochemical, histological, tensile strength and photographic evaluations were done during the study period.

Results: The estimated values of above mentioned parameters on zero day and after healing (18 days) showed significant difference (P<0.05) in relation to collagen content, tensile strength and physical characteristics of wound like extent of wound, size of wound, type of exudates and photography. But clinical, haematological and biochemical data showed no significant difference.

Conclusion: The BM-MSCs were the main pioneers to bring the chronic ulcerative wound towards healing. The procedure is simple, safe and effective in bringing out healing without showing any adverse effect on host.

Key words: BM-MSCs, bovine, chronic ulcerative wound

Introduction

Chronic non-healing or slow healing ulcers represent a major health burden and continue to pose a challenge not only to the patient owners but to the treating physician and medical procedures as a whole. The healing of wound occurs by primary intention and secondary intention [1,2,3]. The therapeutic application of autologous BM derived MSCs (Bone marrow derived mesenchymal stem cells) have revolutionized the field of regenerative medicine. The BM-MSCs were been used for the treatment of chronic wound in diabetic patients [4], for non-healing ulcers of lower extremities in human being [5], cutaneous radiation syndrome in minipig model [6] and in dogs [7] proved successful in their findings. A clinical case of 2 years old Jersey heifer was presented with one large size chronic non-healing wound at lumbar region since 4 months. It was treated with different standard therapeutic regimens since then but showing no tendency towards healing. Hence one clinical trial was made with autologous bone marrow derived stem cell (BM-MSCs) therapy and to know the progress and duration of healing with this application.

Materials and Methods

The owner was fully informed and written consent was obtained about the clinical trial, risk and benefits of the proposed cell based therapy. It was also approved by the Institutional Animal Ethics Committee of the institution since it was a clinical case and related to Agricultural Production Research. Measurement of the wound dimension was taken along with swab from wound bed for bacteriological culture and sensitive test (Fig.1). Biopsy was done from wound bed for histopathological and histochemical study. Wound was washed, cleaned and dressed with fly repellent spray. Under peroneal nerve block and local infiltration with 2% lignocaine hydrochloride, the proposed site i.e antero-medial
aspect of tibia was prepared aseptically for bone marrow collection. A 0.5 cm long incision was made and tibia was drilled with 2 mm drill bit. 10 ml of bone marrow was aspirated in a sterile syringe primed with EDTA (Himedia) @ 1mg/ml. The EDTA mixed bone marrow was despatched keeping inside ice packed thermo cool to the stem cell laboratory for culture and growth.

Conditioned media was prepared for culture with commercially available basic ingredients as per Table-1. The medium was changed regularly and cell morphology was examined under a Nikon phase contrast microscope. After complete colony formation the cultured MSCs were taken for therapeutic application (Fig.2). As the stem cell therapy requires complete sterile medium, the ulcerated wound site was prepared aseptically. The prepared BM-MSCs was diluted with normal saline solution (NSS) at 2: 1 ratio and implanted intra-dermally and topically on wound bed (Fig.3) and then bandaged with paraffin wet bandage. Outwardly fly repellent spray was sprinkled. The clinical parameters like rectal temperature (°C), pulse rate (beats/min), respiration rate (breaths/min) and status of visible mucous membrane were recorded on the day of presentation, BM collection, BM-MSCs implantation and 18th days after healing. The photographic evaluation along with dimension of the ulcerative wound was measured during healing period. Haematological and biochemical parameters were estimated during the study (Table-2). Histopatology with H and E stain and Masson-Trichrome stain for study of collagen content were carried out.

Quantitative estimation of collagen content of tissue was also done with Sircol™ method by help of spectrophotometer at 555 nm wave length [8,9]. The study of tensile strength and strain of tissue was carried out after 60 days of healing and compared with normal skin by help of Universal testing machine (Instron 3382, USA) with running rate 50 mm/minute at 27°C temperature and 65% humidity.

**Results and Discussion**

During the study period the physiological, haematological and biochemical parameters through varied but remained within the normal physiological range. On the basis of culture and sensitivity test antibiotic inj. Ciprofloxacin @ 5 mg/kg body weight was administered parenterally for 5 days. Regarding collection of bone marrow it was collected from femur and tibia of rats [10], mouse [11], mongrel dogs [12], iliac crest of femur [7], humerus in minipigs [6]. In this study the bone marrow was collected from proximal antero-medial aspect of tibia owing to its superficial in position, very thin layer of tissue over the bone and easy for collection. Histopathology after therapy showed neovascularization with appearance of fibroblasts, sebaceous glands and epithelialisation which supports the progression of healing process (Fig.4).

Histo-chemical study showed formation of more collagen content after stem cell therapy. The collagen content with Sircol™ method on zero day, 14th day and 18th day were 11.98 µg/mg, 26.54 µg/mg and 29.24 µg/mg which supports the findings of Ghani et al. [13] regarding wound healing. The tensile strength and
The wound assessment was studied for gross chronic non-healing and ulcerative cutaneous wound healing was assessed by Bose et al. [14] in bovines on 0-3 scale, in horses by Gopinathan et al. [15] on 1-5 scale and in rats by Ramesh et al. [16]. JD and IN designed the study as this is a part of JD’s PhD thesis and IN was his major advisor. PR helped in processing of BM. RKD analyzed the study during healing with that of zero day. The photographs of the post therapy period. SSB helped during collection of wounds were evaluated by three surgeons and showed BM and assisting throughout the study. All authors read and approved the final manuscript.

### Author’s contribution
JD and IN designed the study as this is a part of JD’s PhD thesis and IN was his major advisor. PR helped in processing of BM. RKD analyzed the study during post therapy period. SSB helped during collection of wounds and assisting throughout the study. All authors read and approved the final manuscript.

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### References
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With many stem cell studies currently underway into these areas, it is hoped that these will, one day, become widely used. Spinal Cord Injuries. Research has shown that when paralysed mice are injected with human embryonic stem cells, they have shown the ability to walk. Embryonic stem cells have been used to treat blindness and other consequences of retinal diseases. Researchers utilise the properties of embryonic stem cells to regenerate vision. By transplanting stem cells in sheets over the damaged retina of a patient, the stem cells impart proper function to the eye and restore vision. Jupiter Re: A Rare Side Effects of Stem Cell Therapy: A Case Study My menstrual periods delay in coming while taking stc30 could it be any side effect.. 22 November 2020. A clinical case of 2 years old Jersey heifer was presented with one large size chronic non-healing wound at lumbar region since 4 months. It was treated with different standard therapeutic regimens since then but showing no tendency towards healing. Histo-chemical study showed formation of more collagen content after stem cell therapy. The collagen content with SircolTM method on zero day, 14th day and 18th day were 11.98 Âµg/mg, 26.54 Âµg/mg and 29.24 Âµg/mg which supports the findings of Ghani et al. [13] regarding wound healing. mesenchymal stem cell grafting to treat cutaneous. Pharmaceuticals, Biological and Chemical Sciences, radiation syndrome: Development of a new mini pig. Stem cell therapy, which is widely used nowadays and efficiency-proven, was implemented to a patient having chronic venous insufficiency ulcer. Key Words: Venous ulcer, Stem cell, Stasis. Figure 1: Venous ulcer. In order to treat the underlying venous pathology, the implementation of ligation, ablation and striping of superficial or perforator veins are discussed in surgical topic. Although pentoxifyline has been found to be useful in ulcer treatment, the medical interactions and the side effects of the medicine limits the usability [7]. There is limited number of studies on use of bioengineering-product for skin care, but many products have not been found to be suitable for routine usage. Successful applications of stem cell therapy in diabetic foot ulcers have been reported. The use of cell therapy after traumatic brain injury (TBI) in children can reduce the amount of therapeutic interventions needed to treat the patient, as well as the amount of time the child spends in neurointensive care, according to research by The University of Texas Health Science Center at Houston (UTHealth) Medical School. Our study showed that with stem cell therapy, we need to do less intervention for a shorter period of time for the patient." Explore further. Stem cells linked to cognitive gain after brain injury in preclinical study. Provided by University of Texas Health Science Center at Houston.