Case Report

SUCCESSFUL TREATMENT OF INTER DIGITAL CHRONIC ULCERATIVE WOUND BY MESENCHYMAL STEM CELL THERAPY IN A COW

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Digital disease in cattle is common and the hind digit is most commonly involved (Fubini and Ducharme, 2004). Interdigital over growth in cattle results from proliferations of the subcutaneous tissue and is covered by the hairless integument between the claws (Tyagi and Singh, 2010). These proliferations are many a time get infected with pyogenic organisms producing foul smelling discharge and are converted to chronic ulcerative wound. Now a day's Bone Marrow derived Mesenchymal Stem Cells (BM-MSCs) were found successful for the treatment of chronic wound (Badiavas and Falanga, 2003).

CASE HISTORY AND CLINICAL OBSERVATION

A six years old cross bred Jersey cow was presented with one chronic non-healing wound at interdigital space to the Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Bhubaneswar since four months (Photo 1). Since then it was treated with different standard therapeutic regimens, but there was no tendency towards healing. With the consent of the owner, one clinical trial was made with autologous BM-MSCs therapy .

Photo 1. Photograph showing interdigital hoof lesion.

TREATMENT

The animal was kept fasting for 24 hrs and was sedated with inj. xylazine hydrochloride. Swab was taken from wound bed for bacteriological culture and sensitive test. Biopsy was done for histopathological and histochemical study. Under peronial nerve block and local infiltration with 2% lidocaine hydrochloride, the proposed site i.e proximal antero-medial aspect of tibia was prepared aseptically for bone marrow collection. 10 ml of bone marrow was aspirated in a sterile syringe primed with EDTA (@ 1mg/ml) was despatched keeping inside ice packed thermo cool to the stem cell laboratory of CIFA, Kausalyaganga, Bhubaneswar for culture and growth. Conditioned media (50 ml)was prepared for culture with commercially available basic ingredients as : FBS (Fetal Bovine Serum) 10% , Lonza(5 ml), Sodium Pyruvate 0.1 % Himedia(0.5 ml), NEA (Non essential 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) . The medium was changed regularly and cell morphology was examined under a Nikon phase contrast microscope (Photo 2). After complete colony formation culture MSCs were taken for therapeutic use. The prepared BM-MSCs was diluted with NSS at 2: 1 ratio and implanted intra dermally at different points and also topically on wound bed. It was kept as such for 20 minutes for better adhering to the site and bandaged with paraffin wet bandage. Outwardly fly repellent spray was sprinkled.

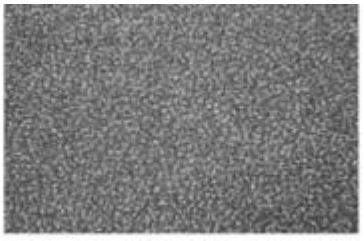

Photo 2. Photomicrograph of cultured BM cells on day-5. 10X

RESULTS AND DISCUSSIONS

The physiological, haematological and biochemical parameters during the study though varies but remained within the normal range. As per culture and sensitivity test inj. ceftriaxone sodium @ 10 mg /kg body weight was administered parenterally for 5 days before stem cell implantation as it required to be implanted in sterile state. There was rapid formation of granulated tissue after BM-MSCs application and the wound was healed after 18 days. The photographs of the wound at different stages upto healing were evaluated and found good healing. The findings of Borena *et al.* (2009) corroborated with the present results. Histopathology with haematoxylin and eosin stain after therapy showed formation of new capillaries which supports the progression of healing process (Photo 3). Histochemical study of tissue with Masson-Trichrome stain was done for collagen content which showed formation of more collagen after stem cell therapy. The quantitative estimation of collagen content with SircolTM assay kit method on zero day, 14th day and 21st day were 10.75 µg/mg, 23.17 µg/mg and 26.85 µg/mg which supports the findings of Singh and Singh (2010) regarding wound healing. Pain free walking distance evaluation showed gradual increase during the study period. Hence the present clinical study shows the autologous implantation of BM derived MSCs is safe, effective and simple procedure for therapeutic efficacy of chronic non-healing wound in bovine.

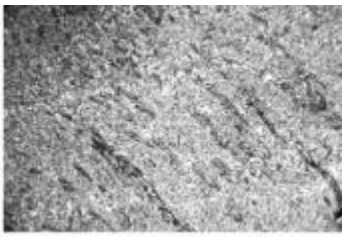

Photo 3. Showing affluent granulation and neovascularisation after MSCs treatment

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