THE EFFECTS OF DIABETES MELLITUS ON FRACTURE HEALING IN BB WISTAR RATS

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Introduction: Diabetic patients suffer an inherent morbidity associated with a delay in fracture healing. Diabetes Mellitus (DM) in streptozotocin treated rats has been associated with impaired fracture healing. This is documented, at least partly by a decrease in cellular proliferation leading to a reduction in collagen synthesis as well as a decrease in callus breaking strength. These characteristics may reverberate even without insulin treatment. The onset of DM in BB Wistar rats is spontaneous and derived from a selective, autoimmune destruction of the pancreatic beta cells. Both genetic and immune factors are associated with this etiology but the precise nature of the disease remains unclear. The BB Wistar rat offers advantages over chemical induction of the disease and currently represents the closest homology to Type 1 DM in man. If the animal is left without insulin death will result due to ketoacidosis. The BB Wistar rat, therefore, serves as the most clinically relevant DM laboratory animal. The purpose of this study was to establish and validate a reproducible femoral fracture model in DM and non-DM BB Wistar rats.

Materials and Methods: Detection and Treatment of DM The BB Wistar rats arrived as either Diabetic Prone (DP) or Diabetic Resistant (DR). DP rats were checked for glycosuria three times a week beginning at 60 days of age. Once glycosuria was detected, tail blood glucose was tested. If the reading was greater than 250 mg/dL, the rat was treated with a palmitic acid, insulin-releasing LINPLANT®. DR rats served as a non-DM control. and received a palmitic acid control implant. Fracture Model. Fourteen days after the implant was placed, a closed mid-diaphyseal femoral fracture was created. Immunohistochemistry: Both DM and non-DM rats were sacrificed at 4, 7 and 10 days post fracture to evaluate cellular mitogenic activity in the fracture callus during the early phases of healing. Specimens were fixed, decalcified, embedded in paraffin and sectioned sagittally through the fracture site. The sections were treated with anti-PCNA/clone PC10, for the presence of the antigen (PCNA), a cellular proliferation marker. Eight specific locations within the fracture callus were analyzed. Positively stained cells were counted at each location under x40 and averaged for each animal. Additional sections were stained with Mallory’s Trichrome to verify the histology of the soft and hard callus. Microscopy: DM and non-DM rats were sacrificed at 4, 6 and 8 weeks post operatively for evaluation of the later phases of healing. Scanning acoustic microscopy (SAM) operates at ultrasonic frequencies and is capable of generating pseudo-color images representing reflected acoustic impedance. These values indicate micromechanical properties. Specimens were fixed, embedded in b(PMMA) and sectioned sagitally through the fracture site. They were scanned to determine the stiffness quality of hard and soft callus. Once scanned, sections were ground and polished and stained with Stevenel’s Blue and Van Gieson Picos-fuchsin for histologic analysis. Statistical Analysis: Overall proliferation and acoustic impedance were analyzed by two-way repeated measures ANOVA with a means contrast used to compare each callus type at different time periods.

Results: General Health: Blood glucose levels in non-DM animals remained within physiologic range at an average value of 90.01±6.96 mg/dL. DM animals exhibited polyuria and glycosuria throughout the duration of the study. These animals gained weight and did not develop ketonuria. Average blood glucose levels remained for both DM and non-DM rats remained between values of 381.99±55.64 mg/dL. Immunohistochemistry: At four days, the fracture site of both DM and non-DM animals consisted of mainly undifferentiated mesenchymal tissue, a large proportion of bone forming cells, and positively stained for PCNA. There was a significant decrease in the number of PCNA positive cells in DM vs non-DM callus (7.0:43±0.33 vs 90.91±7.67; p=0.01). There existed one new bone matrix located between the proliferating periosteal cells and the underlying cortex in both DM and non-DM callus. This region is referred to as the hard callus. At 7 days, substantial amounts of intramembranous bone had formed and cell proliferation in the hard callus had decreased in both DM and non-DM callus but remained higher still in the non-DM (23.94±7.54 vs 62.10±16.65; p=0.0001). Directly adjacent to the fracture site, there appeared fibrous tissue with large areas of a cartilaginous matrix. The amount of cartilage in this region was decreased in the DM rats as well as the distribution of proliferating cells (18.46±9.94 vs 71.50±27.48; p=0.0002) as compared to non-DM controls. At day 10, the number of proliferating cells in the hard callus was further reduced in both groups, but remained significantly lower in the DM animals (16.13±6.20 vs 38.42±10.92; p=0.0007). Cartilage in the soft callus of non-DM animals almost completely replaced fibrous tissue, and contained larger areas of hypertrophic chondrocytes in a more organized columnar fashion. In the DM animals, areas of cartilage were smaller and the size and number of proliferating chondrocytes was greatly reduced (16.00±5.12 vs 46.94±3.92; p=0.01 see Figure 1).

Discussion: Our results show a significant reduction in cell proliferation during the early phases of DM fracture healing which is in agreement with previous work on streptozotocin treated DM rats. This reduction correlates with a decrease in cartilage and collagen synthesis of fracture callus in DM animals also found in previous studies.2 The early deficit in mesenchymal cell proliferation leads to decreased chondrogenesis and impaired endochondral ossification. This study validates the proposed DM fracture model in DM BB Wistar rats that may now be used to evaluate fracture healing adjuncts.


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