THE EFFECT OF WEIGHT-BEARING ON HEALING IN A RAT MODEL OF DISTRACTION OSTEOGENESIS

**Moore, D.C.**; **Ehrlich, M.G.**

**Orthopaedic Research Laboratories, Brown University School of Medicine/Rhode Island Hospital, Providence, RI. Orthopaedic Research Laboratories, 593 Eddy Street, SWP-3, Providence, RI 02903, (401) 444-8904, Fax: (401) 444-6140, douglas_moore@brown.edu**

**INTRODUCTION**

Distraction osteogenesis is a process for lengthening long bones in which the slow, incremental distraction of fracture callus is used to stimulate and prolong active new bone formation. Distraction osteogenesis is used to treat congenital and acquired limb length inequality, post-traumatic segmental bone loss and deformity, difficult fractures, and chronic medical conditions such as osteomyelitis.

As in fracture healing, physiologic loading is considered to be a positive stimulus for healing in distraction osteogenesis. While there is little data on the subject, a recent clinical study has demonstrated that weight-bearing influences the radiologic density of the newly formed callus in distraction osteogenesis,

and laboratory studies have shown that callus volume, mineral content, and fracture strength increase when micromotion is applied during the consolidation phase of limb-lengthening.

We have developed a novel through-knee hindlimb amputation model in the rat to explore the effect of weight-bearing on healing in distraction osteogenesis. We have used the model to explore the influence of weight-bearing on the early biology of healing, as well as on the developing vasculature.

This study was performed to characterize the model and provide a detailed histological description of bone and cartilage formation.

**METHODS**

All procedures involving animals were approved by our institution’s animal welfare committee, which follows the National Institutes of Health guidelines for the humane care and use of laboratory animals.

Under Nembutal anesthesia (Abbott Laboratories, North Chicago, IL), custom unilateral four-pin external fixators were applied to the right femurs of 54 male Sprague Dawley rats (400-425 gm, age < 6 months). After application of the fixators, a mid-diaphyseal osteotomy was performed with a nitrogen powered micro-oscillating saw and the wound was closed in layers. Half of the animals (n = 27) were then made non-weight-bearing via a through-knee amputation of the ipsilateral lower leg. The remaining animals were allowed to bear weight freely. Plain radiographs were obtained post-operatively to verify pin placement and alignment of the bone fragments. Intramuscular analgesics (buprenorphine hydrochloride, Reckitt & Coleman Pharmaceuticals Inc., Richmond, VA) were administered for two days post-operatively, and afterwards as needed. The lengthening protocol involved a seven day latency period, followed by 7.0 mm of lengthening over 21 days (0.17 mm two times per day). Three animals from each group (weight-bearing and non-weight-bearing) were sacrificed at days 1, 3, 7, 10, 14, 21, 28, 35 and 42 post-operatively. The femurs were harvested en bloc, cleaned of excess soft tissue, fixed in cold 10% formalin, decalcified in a solution of formic acid and sodium citrate, and embedded in paraffin. Multiple serial 6.0 µm mid-sagittal sections were cut and stained with either hematoxalin and eosin, Safranin-O/Fast Green, or Masson’s trichrome. The cut and stained slides were examined with an Olympus photomicroscope.

**RESULTS**

**Latency (Days 1 through 7)**

The latency phase in both groups was characterized by organization of the blood clot at the osteotomy site (days 1-3), followed by periosteal hyperplasia and the development of intramedullary fibrous tissue. By day seven, woven bone had begun to form in the periosteum and intramedullary cavity (Figure 1).

**Lengthening (Day 7 through Day 28)**

By day ten (1.0 mm distraction) both groups had a vigorous periosteal reaction with intramembranous new bone formation to the margin of the bone ends, with cartilage formation in the peripheral portions of the callus. The distraction zone contained some intramembranous bone, but primarily fibrous tissue.

By day 14 (2.3 mm distraction) the periosteal reaction had increased and the fibrous tissue in the proximal and distal fragments had begun to align in the direction of distraction. By day 21 the endosteal woven bone at the margins of the bone fragments had also begun to align in the direction of distraction (Figure 2).

**DISCUSSION**

In this study we used a novel through-knee hindlimb amputation model to explore the effect of physiologic weight-bearing on distraction osteogenesis in rats. In a previous study we found that early weight-bearing increased osteoblastic markers (osteocalcin, collagen I, BMP 2,4).

However, in this study we found no substantial difference in the time course or nature of the healing response as assessed by routine histological examination.

**REFERENCES**


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**Figure 1.** Photomicrographs (40x) demonstrating endosteal woven bone in the distraction site at the end of the latency period (day 7) in weight-bearing (L) and non-weight-bearing (R) animals.

**Figure 2.** Photomicrographs (100x) of distraction zone 21 days post-operatively demonstrating columnar intramembranous bone emanating from proximal fragment in weight-bearing (L) and non-weight-bearing animals (R).

**Figure 3.** Photomicrographs (10x) of distraction zone from non-weight-bearing animal 28 days post-operatively (7 mm distraction). The defect is bridged with longitudinally-oriented woven bone and a fibrous interzone.