THE EFFECT OF HINDLIMB ELEVATION ON FRACTURE HEALING:
DECREASED CARTILAGE FORMATION DOES NOT LEAD TO ABNORMAL HEALING

Astronauts involved in space exploration, including interplanetary travel, the construction of orbiting stations, and the maintenance of planet-based facilities, will be exposed to the risk of serious injury including the risk of fracture. This risk flows from the combined effects of a deleterious impact of microgravity on bone mass (decreased bone formation and increased resorption) leading to osteopenia and decreased strength, and the high-risk activities in which astronauts will be engaged. Decreased bone formation in the microgravity environment could also result in the possibility of delayed healing and failure of the bone to return to normal strength at the conclusion of the healing process. To address these issues we evaluated femur fracture healing in rats undergoing hindlimb suspension, a widely used model for studying the effects of space flight on bone.

Materials and Methods

Methods: Hindlimb suspension in the rat is a widely used model for evaluating the effects of mechanical unloading on bone, including the effects of space flight. The rat femur fracture model is commonly used for studying experimental fracture healing. Six-month-old rats have reached skeletal and sexual maturity, while bone physiology in six-month-old rats is comparable to young human adults. Unilateral midshaft femur fractures were made in 180 six-month-old, male, Lewis rats using the Bonnare-Einhorn technique. All studies were approved by the institutional IACUC.

Experimental design: Animals were randomly assigned to one of two groups (90 per group). The day after fracture rats in one group were made non-weight-bearing by hindlimb suspension (HLS), such that their back paws could not touch the cage bottom. The remaining animals were allowed to bear weight normally (WB). Animals were sacrificed at one, two, three, five, seven, and nine weeks after fracture for histology (n=5/group) and at three, five, seven and nine weeks after fracture for mechanical testing (n=15/group). HLS rats were fed ad lib., WB rats were offered food daily equal to the mean amount of food consumed by HLS rats. Weekly radiographs were obtained to assess callus formation and bone bridging.

Results

Radiographs: Evaluation of weekly radiographs showed progressive callus formation in both groups. Calluses in WB animals were larger than in HLS animals at all times after fracture (Figure). Bone bridging was seen earlier on radiographs of HLS animals: at three weeks 60% of calluses in HLS animals were bridged compared with none in the WB group; at five weeks bridging was seen in all radiographs of HLS animals and in 70% of WB animals. At seven and nine weeks all HLS fractures appeared healed on radiographs; one nonunion was seen in the nine week WB animals.

Mechanical testing: Two non-unions were seen: one at three weeks (HLS) and one at nine weeks (WB). Force to failure, energy absorption and stiffness were equal in the two groups seven and nine weeks after fracture (Figure). Energy absorption was significantly increased in WB animals at three weeks, while force to failure and stiffness were significantly increased in HLS animals at five weeks.

Histology: subperiosteal bone formation, chondrogenesis, and endochondral ossification were seen in all calluses two and three weeks after fracture (data not shown). Five weeks after fracture there was bone bridging the fracture site in all HLS rats but in none of the WB animals. In WB animals the gap between the bone ends was filled with cartilage (red on Safarin-O staining) or a combination of fibrous and cartilage tissues (data not shown). By nine weeks after fracture endochondral bone formation bridged the fracture gap in all WB animals. The smaller fracture calluses in HLS animals was associated with decreased formation of subperiosteal bone and cartilage. Endochondral replacement of the smaller cartilage mass was completed earlier in calluses from HLS animals (data not shown).

Discussion

Relatively equal mechanical properties seven and nine weeks after fracture, together with bone bridging on histology, are consistent with complete healing of fractures in both treatment groups.