A simulated weightlessness state diminishes cortical bone healing responses

R.J. Midura, X. Su, C. Androjna

Department of Biomedical Engineering and Orthopaedic Research Center, Cleveland Clinic, Cleveland, OH, USA

Keywords: Weightlessness, Bone Healing, In vivo Micro-CT Imaging, Osteoprogenitor Colony Forming Units

A state of chronic immobility resulting from extended spaceflight, bed rest or paralysis can present severe secondary complications for individuals confronted with long bone cortical fractures that fail to heal. These secondary complications can generally lead to a decreased quality of life and function for these individuals, and may potentially lead to a life-threatening situation. One potential cause of these failed fracture healing situations may be the prolonged state of non-weight-bearing (NWB) that chronically immobile individuals are exposed to. The research investigation of this clinical issue has been hampered to some extent by the inconsistent use of pre-clinical animal models of chronic immobility to study cortical bone fracture healing under a physiologically relevant NWB condition. In this brief report, we will provide some very recent pre-clinical findings concerning cortical bone healing in a simulated weightlessness rodent model exhibiting a physiologically relevant NWB condition in its hind limbs. Specifically, we will document that this rodent model of a NWB situation mimics the human condition of chronic immobility with respect to the high incidence of delayed or failed healing of cortical bone fractures. Further, we will provide evidence to substantiate a hypothesis that a long-term NWB condition acts to impair cortical bone healing by diminishing the number of functional bone progenitor cells at the repair site required to heal bone fractures.

Data from our laboratory, and other previously published reports in the literature, demonstrate that cortical bone healing in a rat NWB model (hind limb disuse resulting from tail suspension) mimics the diminished bone regeneration response in humans exposed to a prolonged state of NWB. Standardized bone trauma (0.2-mm wide osteotomies) was introduced into the fibulae of full weight-bearing (WB) and NWB rats (Figure 1), and the bone healing response monitored longitudinally via micro-computed tomography imaging. We found that a mid-diaphyseal, 0.2-mm osteotomy in the fibulae of NWB rats exhibited an inferior healing response over a 5-week period as compared to full weight-bearing (WB) control animals (Figure 2). Specifically, NWB healing responses yielded poor hard callus formation that resulted in significantly decreased hard callus size, extent of bridging callus across the trauma site, and bridging callus strength as compared to full WB counterparts. Histological examination of the healing callus showed an inad-

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The authors have no conflict of interest.

Corresponding author: Ronald J. Midura, Ph.D., Cleveland Clinic, Dept. Biomedical Engineering, ND20, 9500 Euclid Ave., Cleveland, OH 44195, USA E-mail: midurar@ccf.org

Accepted 30 July 2006

Figure 1. Osteotomy trauma models a simple, transverse cortical bone fracture pattern. Diagram illustrates the width ➊, placement ➋, and angle ➌ of the fibular osteotomies in Sprague-Dawley rats. Lower graphs show combined data from left and right fibulae of 8 rats (mean±SD).

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equately mineralized extracellular matrix surrounding the initial trauma site, suggesting impairment in osteogenesis.

One hypothesis to explain the underlying mechanism of this NWB-mediated impairment of osteogenesis at a cortical bone trauma site states that the local osteoprogenitor cell populations having access to this damage site in NWB animals are decreased as compared to those of full WB animals. To test this hypothesis, marrow-derived, connective tissue progenitor cells (CTPs) were recovered from the tibiae of the same hind limbs that provided the osteotomized fibulae used to assess bone-healing responses. In rodents, the tibia and fibula share marrow tissue through a complete synostosis between these bones, thus substantiating this experimental approach. CTPs were isolated from identical-volume marrow aspirates using a conventional colony-forming unit assay, and osteoprogenitor cells were identified in these assays by intense staining for alkaline phosphatase (AP) activity using a commercial histochemical assay kit (Vector Red, Vector Labs, Burlingame, CA). A key distinction of our CTP-AP+ (or CFU-Ob) assay from previous studies is that the CTPs were allowed to grow for only a 6-day period in a growth medium that does not induce further osteogenic differentiation beyond the level already present in situ at the time of marrow tissue isolation. Thus, our current CTP-AP+ assay attempts to measure the number of existing osteoprogenitor cells in vivo at the time of marrow tissue recovery.

The total number of CTPs recovered from NWB marrow tissue was only 24% of that recovered from WB marrow tissue [NWB= 10±5 CFU/4-cm² as compared to WB= 42±12 CFU/4-cm²; p<0.05, ANOVA, n=4]. Furthermore, the numbers of CFU-AP+ were proportionally reduced in NWB as compared to WB rats. These results indicate that a state of prolonged NWB in the rat hind limb leads to a diminished population of marrow-derived, osteoprogenitor cells in the long bones of these NWB limbs. These findings are consistent with previous reports by other groups⁶, and support our stated hypothesis that one underlying mechanism to explain an NWB-mediated impairment of osteogenesis at a cortical bone trauma site involves a decreased population of local osteoprogenitor cells having access to the damaged bone site that would be involved in the regeneration process.

Thus, a state of chronic NWB resulting from extended spaceflight, bed rest or paralysis leading to a failed cortical bone healing response may be the result of the adverse effects of a prolonged state of non-weight-bearing on the local population of bone progenitor cells at the fracture site. A decreased population of reparative cells at the local fracture site will likely diminish the rate of bone tissue regeneration, and contribute to the delay in re-establishing the mechanical continuity of the long bone’s cortical shaft.

Future investigations are underway attempting to improve the bone healing response in NWB animals using intermittent parathyroid hormone therapy, and to determine the effects of such therapy on the local bone progenitor cell populations in such treated bones. Ultimately, our long-term goals focus on identifying agents and approaches that will reverse the outcomes of diminished cortical bone healing responses in non-weight-bearing animals.

Acknowledgement

This study was funded by a grant from the National Space Biomedical Research Institute, NASA, Project number BL00405.

References