A Novel Model for Inducing Joint Inflammation and Degeneration with a Pharmacological Intervention to Reduce Its Effects

Introduction:
Several animal models for osteoarthritis have been developed and their degenerative progression is similar to humans, but the initiating factors are not representative of the general population. An ideal model would involve the interaction between inflammation and biomechanical changes observed in humans without surgical trauma, targeted genetic manipulation, or chemical induction. This study assessed a voluntary high-repetition, high-force (HRHF) task in rats that required one limb to reach for, grasp, and pull a handle while the contralateral limb provides postural support. The high demand tasks lead to inflammation, fibrosis, and degenerative changes in nerve, muscle, tendons and bones (1-3). However, the influence of this task on joints has not been assessed.

Ibuprofen, a common nonselective nonsteroidal anti-inflammatory drug, was used as a therapeutic intervention in this study. Ibuprofen’s role in osteoarthritis is unclear. Its therapeutic effect should result in a chondroprotective effect but prior research has suggested that it induces catabolic changes. Additional research is needed to determine the role of ibuprofen in osteoarthritis. The purpose of this study was to evaluate the potential of a voluntary HRHF task to induce joint inflammation or degeneration and to determine the effect of ibuprofen on these outcomes.

Methods:
A randomized controlled trial design was used. To address our goals, we used: (a) biochemical analyses to assess joint inflammation, (b) histological analyses to assess joint degeneration, and (c) biochemical analysis of a serum marker of collagen degradation - each with or without ibuprofen treatment.

83 young adult, female Sprague-Dawley rats were used. Animal care and use was monitored by the University Animal Care and Use Committee to assure compliance with Federal and NIH regulations. Experimental rats were trained to reach forward to pull a handle at a rate of 12 reaches/min at 60±5% of maximum voluntary grip force for 2 hrs/day in 30 min sessions, 3 days/wk for up to 12 weeks. Both preferred reach and support limbs were analyzed. Animals were divided into 5 groups, including 2 experimental groups: 1) rats that performed a HRHF task without ibuprofen for 6 or 12 weeks (HRHF6 or HRHF12); 2) rats that performed a HRHF task for 6 or 12 weeks with ibuprofen treatment (HRHF6+IBU or HRHF12+IBU). Ibuprofen treatment was initiated at week 4 of task performance (liquid Motrin given daily in drinking water; 45 mg/kg body wt). Three groups did not perform the task and served as controls: trained controls without or with ibuprofen (TR CON or TR CON+IBU), and normal controls (NORM).

Biochemical analysis of joint inflammation was assessed in homogenized wrist joints and radioulnar diaphyses collected from HRHF6 (n = 5), HRHF12 (n = 6), HRHF6+IBU (n = 5), HRHF12+IBU (n = 6), and NORM (n = 9) rats. Three pro-inflammatory cytokines (Interleukin [IL]-1α, IL-1β, tumor necrosis factor [TNF]-α) and an anti-inflammatory cytokine (IL-10) were assayed by ELISA. Data were normalized to total protein concentrations.

Histopathological scores were assessed in paraffin embedded and sectioned joints stained with Safranin O and fast green in HRHF12 rats (n = 15). Histopathological articular cartilage of the distal radius was assessed using the modified Mankin Scoring System (4), which evaluates structure, cells, and saturation of safranin staining indicative of proteoglycan content in subscales. Immunohistochemistry was performed to qualitatively assess the presence of ED1+ cells (e.g., macrophages, osteoclasts, and their progenitors) in NORM, TR CON, HRHF12, and HRHF12+IBU. To validate the histological findings, serum levels of the C-terminal of peptide generated by cleavage of types I and II collagens by collagenases (C1,2C) via ELISA. C1,2C serum levels reflect degradation of tendon, bone, and articular cartilage. Serum was collected from HRHF6 (n = 5), HRHF12 (n = 6), HRHF6+IBU (n = 7), HRHF12+IBU (n = 6), TR CON+IBU (n = 6), and NORM (n = 6) rats.

ANOVA analyses (p ≤ 0.05) were performed and Bonferroni posthoc tests.

Results:
All four cytokines were elevated in HRHF12 rats compared to the other groups regardless of limb or region. Also, IL-10 was significantly higher in HRHF12+IBU rats compared to NORM. Histopathological total scores of joint degeneration were significantly greater in HRHF12 rats (Fig 1-A). Histopathological total scores of joint degeneration were significantly greater in HRHF12 rats. The HRHF12 group had a greater loss of safranin staining in the epiphyseal plates than the two medicated groups. A reduction in safranin staining was also observed in the articular cartilage of HRHF12 rats compared to the other groups (Fig. 1-B). Other cartilage changes in the HRHF 12 rats included irregular surface and chondrocyte proliferation (cloning), with a few also showing pannus and hypocellularity (cell loss). The highest scores were noted for the radial side of the radius in these rats. HRHF12 joints contained ED1+ cells, most likely osteoclast progenitor cells, in the subchondral bone of the distal radius and carpal bones. ED1+ macrophages were also increased in HRHF12 synovium. No ED1+ cells were seen in TR CON or HRHF12+IBU joints. C1,2C serum concentrations were significantly reduced in the ibuprofen treated groups than in the no ibuprofen groups.

Discussion:
The HRHF task induced joint inflammation and degeneration after 12 weeks. The histopathological articular cartilage were indicative of early joint degeneration (e.g., decreased proteoglycan staining with minimal structural changes; higher histological scores in the radial region). Ibuprofen had anti-inflammatory and chondroprotective effects. This rat model may be a successful model to assess early osteoarthritides and future disease modifying osteoarthritis drugs.

References: