Nsaid Treatment Does Not Disrupt Intervertebral Disc Matrix
Glycosaminoglycan Production In Vitro Or In Vivo

Tiffany Kadow¹, Pedro Pohl², Takashi Yurube³, Robert Allen Hartman, MS ⁴, Kevin Ngo ⁵, James D. Kang, MD ⁶, Gwendolyn A. Sowa ².
¹University of Pittsburgh Medical Center, Pittsburgh, PA, USA, ²UPMC, Pittsburgh, PA, USA, ³University of Pittsburgh, Pittsburgh, PA, USA.

Disclosures:

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDS) are commonly recommended medications for patients with musculoskeletal complaints. Despite the high prevalence of use, there is little literature to support the safety of these medications on the intervertebral disc matrix. The literature which is available is largely based in articular cartilage studies and suggests that NSAIDS decrease glycosaminoglycan (GAG) synthesis, a crucial component of the intervertebral disc matrix. Given the prevalence of use and lack of evidence regarding the safety of NSAIDS on the unique environment of the intervertebral disc, this study aimed to demonstrate the impact of indomethacin on glycosaminoglycan contents both in vitro and in vivo use of NSAIDS.

Methods: Annulus fibrosus cells from skeletally immature New Zealand white rabbits were harvested, isolated, expanded, and plated. They were exposed to indomethacin 10nM for 24 hours and then GAG content was evaluated via dimethyl methylene blue (DMMB) assay (n=3). In vitro evaluation of GAG content was standardized to DNA amount as measured by PicoGreen assay. In vivo experiments were conducted with 6 skeletally mature New Zealand white rabbits. All underwent surgical annular puncture at L2-3, L3-4, and L4-5 with puncture being achieved via a lateral surgical approach to the spine and puncture with a 16g needle to a depth of 5mm as previously reported to model chronic degenerative models 3. Half (n=3) were exposed to 6mg/kg of oral indomethacin, the metabolic equivalent to human dosing 2, while the other half were not given any additional treatment and served as the control (n=3). Animals were treated for a total of 12 weeks and then sacrificed. Unpunctured intervertebral discs T11-12 and T12-L1 were removed and analyzed via DMMB assay for total glycosaminoglycan content and assess the changes on the disc from indomethacin alone. Annulus fibrosus tissue was standardized to both weight as well as DNA content via PicoGreen assay, while nucleus pulposus tissue was standardized to DNA content alone due to the small amount of tissue available. Sagittal T2 MRIs (3mm slices) were obtained at 0 and 12 weeks via a 3 Tesla Trio (Siemens) MR scanner. Animals were sedated with for imaging to provide for quality images. MRI index (the signal intensity of the nucleus pulposus divided by the nucleus pulposus area) was measured as an overall sign of disc health (MATLAB software, Natick, MA).

Results: Early investigations demonstrated that annulus fibrosus cells were able to achieve 100% survival at a maximum concentration of 10nM, therefore this was used for the in vitro experiments. These in vitro studies demonstrate a 2.1-fold increase in total proteoglycan content after exposure to indomethacin 10nM for 24 hours. In vivo analysis of annular fibrous tissues exposed to indomethacin for 12 weeks had a 1.5-fold increase in total proteoglycan content and nucleus pulposus cells had a 1.25-fold increase in proteoglycan content. MRI index was nearly equivalent at 38% for rabbits treated with and without indomethacin.

Discussion: Previous work in articular cartilage has demonstrated detrimental effects of NSAIDS on matrix components through decreased production of matrix proteins such as collagen synthesis and production of proteoglycans. In order to evaluate for the most potentially harmful outcome, indomethacin was selected given the previous evidence suggesting its potent toxic effects on articular cartilage. While prior evidence might suggest that indomethacin is matrix toxic, annulus fibrosus cells appeared to thrive with NSAID treatment in vitro. Greater evidence was developed in the in vivo study which demonstrates the impact of this medicine at a dosage and exposure to cells would be manifested in patients. Furthermore, retention of the MRI index between both treated and untreated rabbits indicates maintenance nucleus pulposus, which is primarily composed of glycosaminoglycans.

Significance: Our in vivo study also demonstrates the long term effect of chronic NSAID treatment on the intervertebral disc matrix.

Acknowledgments: Acknowledgements: We gratefully thank the support of the CSRS Resident/Fellow Grant and the department of Orthopaedic Surgery at the University of Pittsburgh

References:
6. Ding C. Do NSAIDs affect the progression of osteoarthritis? Inflammation. 2002;26:139-
Nucleus Pulposus - GAG

STAB + IND

STAB ONLY
Puncture only
0wk-12wk

Indomethacin + Puncture
0wk – 12wk

ORS 2014 Annual Meeting
Poster No: 1650