**INTRA-ARTICULAR INJECTIONS OF HIGH MOLECULAR WEIGHT HYALURONAN PREVENTS SYNOVIAL HYPERPLASIA AND CARTILAGE DEGENERATION IN POST-INJURY MURINE KNEE JOINTS**


*University of South Florida, Tampa, Florida; **Rush Medical University, Chicago, IL

**wanemaet@health.usf.edu**

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**Introduction:**

Intra-articular hyaluronan injections are widely used for the treatment of pain and stiffness in human OA. The therapeutic effects have commonly been attributed to improved joint lubrication and improved biomechanics. On the other hand, the possibility that the benefits of HA therapy results from cell-mediated responses including anti-inflammatory and pro-anabolic changes has also been examined with chondrocyte cultures (refs 1-4), however the cellular and molecular mechanisms underlying such putative receptor-mediated changes remains largely unknown. In this regard, an increasing number of HA receptors have been cloned including CD44, RHAMM, LVE1 and more recently TLR2 and 4. All of these receptors, with the exception of LVE1, are transmembrane signaling molecules which respond to HA ligation with changes in cell proliferation, migration, phagocytosis and apoptosis. For example, binding of high molecular weight HA to TLR2/4 results in an NFkB-mediated anti-apoptotic effect in epithelial cells (ref 5).

To investigate the possible receptor/signaling pathways involved in the anti-inflammatory effects of intra-articular HA, we have utilized a murine model of TGF-b-stimulated synovial hyperplasia and cartilage degeneration. A single post-injury HA injection reduces synovial hyperplasia, osteophyte formation and cartilage erosion in this model, setting a framework for in vivo analysis of cell-mediated responses to intra-articular HA.

**Methods:**

Mice (C57Bl/10, male, 12 wks) received 2 injections of HrTGFb (BenderMed Systems), on alternate days as described by van Beunigen et al (Ref 6). One day after the last injection, one group of animals received a single dose of HA (10 ul of 5mg/ml Supartz in PBS), a second group received 10 ul of PBS and the third group remained untreated. Animals (n = 6 per group) were sacrificed at 1 and 7 days after HA or sham injections, hind limbs and major organs were harvested and prepared for histological evaluation by H/E, Saff-O and IHC for macrophage (F4/80) and granulocyte (GR-1) cell-surface markers. Blood was collected from the submandibular vein at 4 day intervals, and plasma analyzed for cytokines and chemokine levels by Luminex Microarray using the Mouse2/9 Plex and 23-Plex platforms. Statistical analyses were performed with MANOVA.

**Results and Discussion:**

Thorough statistical analysis of serum cytokine data was difficult secondary to small sample size. However, statistically significant changes were noted in the following cytokines: IL-17 and TNF-alpha decreased in the TGF-beta group from baseline to day 1 post injection, GM-CSF and MCP-1 decreased in the TGF group from baseline to Day 7 post injection, IL-12(70) increased in the TGF + HA group from baseline to day 1 post injection, and KC decreased in the TGF + HA group from baseline to day 7 post injection. Other cytokines demonstrated trends, but did not show statistical significance due to insufficient power. This data seems to suggest a systemic response that needs to be further explored with a larger sample size.

TGFb injection resulted in hyperplasia of both the synovial and periosteal lining at day 1. This was followed by osteophyte formation and cartilage lesion at week 1 (Figure 1). Immunohistochemical staining (at day 1) for the macrophage marker (F4/80) showed an induction of positive cells in the synovial lining, the periosteal lining and also, rather surprisingly, in the cartilage mid zone. These changes were also evident at 1-week post-TGF injection. There was however no evidence of granulocyte (GR-1 marker) recruitment to the treated knee joint.

Intra-articular treatment with HA (after TGF-beta injection) reduced the synovial hyperplasia, minimized osteophyte formation and prevented cartilage lesion development. Interestingly, the treatment with HA also resulted in a robust infiltration of granulocytes into the peri-mesenchymal synovial lining at day 1 and there was an associated reduction of F4/80 positive cell numbers in all tissues in the joint (Figure 2). Taken together this suggests that the HA is capable of modulating the local innate immune response (GR-1 induction and F4/80 blockade) to tissue injury. This observation may provide a novel explanation for the reparative effects attributed to HA joint therapy in human OA. Analysis of human joint tissues for F4/80 and GR-1-positive cells, before and after HA therapy, will be required to validate the clinical significance of these findings.

**References:**


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