A Comparative In Vitro Study on the Effects of Various Concentrations of Low and High Molecular Weight Hyaluronic Acid on Human Chondrocyte Cell Metabolism

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Summary:
Hyaluronic acid is a conservative treatment modality in osteoarthritis, our study investigates its effects at a cellular level in human chondrocytes to help better clinical treatment

Abstract:
Osteoarthritis is the most common form of arthritis in the world with more than one third of the world’s population suffer from this disease. The disease symptomatology reduces the subject’s quality of life drastically in otherwise healthy individuals. Many treatments have been explored from more conservative oral medications to surgical procedures to try delay the onset of osteoarthritis.

Hyaluronic acid (HA) has been used as a treatment modality for over two decades in humans with research in horse intra articular injections dating back to 1969.

Clinical results have been ambiguous at best and advantage of the treatment has not been determined. Animal studies using bovine articular cartilage in vitro showed greater chondrocyte DNA proliferation with low concentrations of HA as opposed to those without HA or higher concentration.

We aimed to determine the effect of concentrations of high molecular weight(HMW) and low molecular weight (LMW) HA on human chondrocytes in vitro, so as to ascertain any potential benefit or toxicity the drug may have. This will aid in our use of the drug in clinical practice and lead to better patient outcomes.

Materials and Methods

Tissue Culture and Harvest
All procedures were approved under the institutional ethical committee and with patient informed consent. Three cartilage bone plugs were harvested during total knee arthroplasty from patients suffering from osteoarthritis scheduled for surgery under sterile conditions.

Chondrocytes were isolated and cultured to passage one, three samples from passage one were cultured to passage two. Both passage chondrocytes were seeded in T-25 flasks with concentrations of 0.1 mg/ml, 1mg/ml & 2 mg/ml HMW and LMW HA against control. Regular HA medium changes were done and cells harvested on day 14 for assessment.

Cell viability: after necessary slide preparation using a hemocytometer cells were counted in 4 squares under 100X magnification. Stained cells (non viable) and non stained cells (viable) were counted and made into a percentage.

Cell count: assessed using a coulter counter after slide preparation.
CD44+ expression: measured using flowcytometry (CD44+ expression indicates hyaluronan interaction with the cell)

Statistics:
One way anova test was used to compare the effects of the concentration in the low molecular weight (LMW) and high molecular weight (HMW) hyaluronic acid groups
Independent T test was used to assess compare the effects between the concentration in LMW and HMW HA groups.

Results:
As the concentration of both HMW and LMW HA increases, there is decrease in cell viability count and CD44+ expression in both passage 1 and 2 but the decrease is not statistically significant. However, the decrease in CD44+ count in HMW HA passage 1 is significant (p value=0.04).

Conclusion
From our results it is evident increasing concentrations of both HMW and LMW cause a decrease in cell viability, cell count and CD44+ expression. Our study indicates the use of HA to promote chondrocyte proliferation and aid in treatment of osteoarthrosis is not a viable treatment modality.