THE EFFECTS OF SELENIUM AND THE FUNGAL MYCOTOXIN NIVALENOL ON CARTILAGE MATRIX METABOLISM: A CULTURE SYSTEM TO MIMIC PATHOLOGY IN KASHIN-BECK DISEASE.

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Introduction
Kashin-Beck disease (KBD) is a special endemic osteoarthropathy with pathological changes of degeneration and necrosis occurring in the growth plate and articular cartilage of human limbs and joints. It clinically manifests with stiffness, pain, a decrease in range of movement and therefore deceased function. The geographic distribution of KBD is mainly in a broad belt across China, Tibet and parts of Eastern Russia, and in endemic areas affects up to 90% of the population. Children are affected after the age of 5, and the ankle is the most common joint involved.

It has been postulated that KBD is due to the combined effect of a regional deficiency of dietary selenium, and the infiltration of Nivalenol (a fungal mycotoxin) into food stores.

We have developed an in vitro system in which chondrocytes are cultured in media supplemented with selenium (SEL), nivalenol (NIV) or in combination, to produce an ex vivo cartilage graft in an attempt to reproduce pathological changes found in KBD and better understand the cellular and molecular mechanisms underlying its pathogenesis.

Methods
Articular cartilage from 7 day old bovine metatarsophalangeal joints was enzyme digested in 0.1% pronase (3 hours) and 0.04% collagenase (overnight) to release chondrocytes. Cells were washed and seeded at high density in Millicell filter inserts (12mm diameter; Millipore, MA) coated with type II collagen and maintained for 4 weeks in DMEM supplemented with 20% heat-inactivated FBS, ascorbate (100µg/ml) and TGFβ3 (5ng/ml), and additionally supplemented with either selenium selenite (Sigma), Nivalenol (Sigma) or both at concentrations of 0.01, 0.05 and 0.1µg/ml. Media was refreshed thrice weekly and stored for analysis.

The cartilage grafts were harvested after the 4 week culture period, weighed and extracted in 4M guanidium chloride containing an inhibitor cocktail. Residues were digested with papain. DMMB assays were used to determine the glycosaminoglycan (GAG) content in all media samples, guanidine extracts and papain digests.

SDS PAGE and Western blotting with a panel of monoclonal antibodies recognizing chondroitin sulphate (CS), keratan sulphate (KS) and protein core epitopes was used to determine aggrecan and GAG composition, and size exclusion chromatography carried out to ascertain if GAG content was in the form of aggregate or monomer.

Previous work examined graft histology with alcian blue staining for proteoglycan content, before counterstaining with haematoxylin, and graft tissue thickness with image analysis.

Results
In those grafts supplemented with higher doses of NIV (0.05 and 0.1µg/ml), there was a substantial reduction in the total GAG synthesized over the 4 week period. To a lesser extent, high doses of SEL also had the same effect.

Nevertheless, the amount of GAG released into the media remained fairly constant between the treatment groups, with the marked reduction seen in the guanidine extracts of the cartilage grafts (see figure 1). Of the GAG synthesized over the 4 week culture period, for controls, SEL at all doses and NIV at 0.01µg/ml, the percentage of GAG incorporated as a graft was approximately 65-75%. However, in the cultures treated with NIV at 0.05 µg/ml the percentage incorporated into a graft was reduced to 50%, and indeed at 0.1 µg/ml only 10% was incorporated, and this was only papain extractable.

Western blot analysis with a series of antibodies recognizing CS isomers on guanidine extracted aggrecan showed that controls, SEL and lowest dose NIV treatments all had high molecular-weight core protein. However, the doses of NIV at 0.05µg/ml demonstrated multiple banding with 2B6 and 1B5 suggestive of cleavage. Further analysis demonstrated that KS was reduced in NIV treated cultures and appeared to contain less CS substitutions on the aggrecan core protein.

The majority of aggrecan present in both the media and guanidine extracts was in the aggregated form.

Figure 1. Total GAG content in grafts for each treatment, demonstrating little present in the matrix of those treated with higher doses of NIV

Discussion
Grafts treated with NIV show an inability to retain GAG within the cartilage extracellular matrix, but Western blot analysis demonstrated only minor changes in the composition of the aggrecan in relation to protein core length and CS/ KS side chain substitutions or length, suggesting that it is the overall quantity of GAG synthesis that is affected and not the quality. The results of adding SEL suggest that we are yet to find the ideal concentration to supplement the grafts with, or that it has its protective effect on in vivo cartilage via an indirect pathway.

The results of size exclusion chromatography show that the majority of aggrecan was present in an aggregated form, with only a small proportion present as monomer, regardless of the treatment the graft was subjected to. This suggests that there was a sufficient amount of hyaluronan and link protein in all grafts.

Collectively our data suggests that the cartilage pathology in Kashi-Beck disease results from a decreased synthesis of aggregated aggrecan and possible alteration in cartilage collagen metabolism, that results in inability for the cartilage proteoglycan aggregates to remain entrapped within the tissue extracellular matrix.

This aberration in proteoglycan and collagen metabolism compromises the physiological function of the tissue of resisting compressive loads during joint articulation.

References

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