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A study on keratan sulphate as a molecular marker to monitor cartilage metabolism in horses

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Joint damage is difficult to estimate its term of severity and extent objectively. Much damage may be done early in the disease process, before the detection of any radiographical changes, although it is uncertain which of the many aspects of synovitis are responsible for joint damage. Sensitive ways of assessing the current status of cartilage and bone changes during the early inflammatory joint disease would be of potential value to clinicians for more accurate diagnosis and precise treatment.

Articular cartilage is a specialized tissue composed of mesenchymal cells and chondrocytes, embedded in an extensive extracellular matrix. Proteoglycan molecules are usually rapidly degraded into smaller fragments, which are either engulfed by the chondrocytes or diffused into the body fluids that are in contact with cartilage. Consequently, proteoglycan fragments and glycosaminoglycans are found in the synovial fluid, and several species of glycosaminoglycans, including chondroitin sulphate and keratan sulphate, are also detected in the blood or urine. Keratan sulphate is a glycosaminoglycan that is distributed in the extracellular matrix of joint cartilage. Keratan sulphate is mainly found in aggrecans (approximately 99%), and to a lesser extent, in the cornea and other tissues. Therefore, to estimate the total catabolic activity of cartilage, keratan sulphate could be used as a representative metabolite of carti-

lage in the synovial fluid and blood.

In this study, firstly, a sensitive and specific monoclonal antibody to equine keratan sulphate, 1/14/16H9, was established to develop an enzyme immunoassay for measurement of the concentration of keratan sulphate in serum and synovial fluid from horses. An inhibition enzyme linked immunosorbent assay (ELISA) was optimized by using the established monoclonal antibody, and keratan sulphate concentration in 18 synovial fluid and 48 serum samples obtained from clinically normal horses and horses with arthritis was measured. Precision data were obtained over the range of 10 to 160 ng/ml. The within-and between-assay coefficients of variation of the assay were 10.0 and 12.7%, respectively.

Secondly, the significance of keratan sulphate reacting to the newly-established anti-equine keratan sulphate 1/14/16H9 antibody and an anti-human keratan sulphate 1/20/5D 4 antibody as a marker of cartilage metabolism was evaluated by using an in vitro model of equine articular cartilage. Articular cartilage was harvested from clinically healthy 6 month-old Thoroughbred foals. Articular chondrocytes were centrifuged and cultured as pellets in centrifuge tubes. Chondrocytes were incubated with insulin-like growth factor (IGF)-Ia, transforming growth factor (TGF)-b1 or interleukin (IL)-1a for 2 weeks. The sulfated glycosaminoglycans (GAG) and keratan sulfate concentrations in pellets or

media were measured by a 1,9-dimethyl-methylene blue colorimetric assay and an inhibition ELISA, respectively. The concentration of GAG in the culture media was significantly increased by the supplement of IGF-Ia and TGF-b1. The concentration of GAG in pellets was significantly increased by the supplement of IGF-Ia, but not by TGF-b1.

The concentration of keratan sulphate reacting to 1/14/16H9 and 1/20/5D4 antibodies in the culture media were also measured. A high correlation between GAG and keratan sulphate concentrations was found in the media of pellets stimulated by IL-1a ($r=0.87$ and 0.84 ; GAG to 1/14/16H9 and to 1/14/16H9 keratan sulphate, respectively), but lower in those stimulated by IGF-Ia ($r=0.43$ and 0.59 ; GAG to 1/14/16H9 and to 1/14/16H9 keratan sulphate, respectively).

These two differently reactive keratan sulphate, 1/14/16H9 and 1/20/5D4, concentrations in serum and synovial fluid could contribute as a marker to understand principally the catabolic process of articular cartilage metabolism and also represent in part some aspects of the cartilage anabolic process.

Finally, the clinical significance of keratan sulphate as a cartilage metabolic marker was evaluated by measurement of keratan sulphate concentration reacting to 1/14/16H9 and 1/20/5D4 antibodies in serum and synovial fluid from horses.

Serum keratan sulphate concentration in growing foals (up to 3 months of age) was significantly higher than the value in foals over 4 months old. Serum keratan sulphate concentration in three foals, which had joint abnormalities, was higher than those in the normally growing 12 foals during the first 3 months after birth.

In on-training horses, keratan sulphate concentration in 2-year old horses was significantly higher than 3- or 4-year old horses.

Significantly higher concentration of keratan sulphate was found in the on-training group than in the long-term resting group in 2-year old horses. Immediately after the training in healthy horses, keratan sulphate concentration was significantly higher than before the training.

The concentration of keratan sulphate in sera peaked 3 days after the experimental injection of chymopapain intra-articularly, and was approximately 1.9-and 1.4-folds higher than the preinjected value, in 1/14/16 H9 and 1/20/5D4 keratan sulphate, respectively. The concentration of keratan sulphate in the synovial fluid peaked 3 days after injection of chymopapain, and was approximately 4-folds higher than before the injection, in both keratan sulphates. There was no significant difference in the levels of keratan sulphate concentration reacting to 1/14/16H9 and 1/20/5D4 antibodies in synovial fluid between normal horses and horses with osteoarthritis.

Both established 1/14/16H9 antibody and the common antibody (1/20/5D4) for the detection of keratan sulphate could contribute to understand the catabolic activity of joint cartilage in the various conditions of the joints in horses.

In conclusion, my study showed that the measurement of keratan sulphate reacting to 1/14/16H9 and 1/20/5D4 antibodies in sera and synovial fluid would be of value to understand the cartilage metabolic activity related to various conditions of the joints. Comparison of the concentrations of keratan sulphate reacting to both newly established 1/14/16H9 and the common antibody 1/20/5D4 would contribute to understand more accurately the catabolic or anabolic activity of joint cartilage in the various conditions of the joints in horses. The measurement of keratan sulphate in sera and synovial fluid would be of value to

understand the catabolic activity of joint cartilage, so that keratan sulphate could be an noninvasive and possibly direct informative

marker to detect cartilage pathology in horses with joint diseases.

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Pathomorphological studies of cholangiohepatitis in broiler chickens

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Enlarged, discolored and firm livers, often associated with cholecystitis and extrahepatic bile duct distended with bile, have been reported in broiler chickens. Histopathologically, these livers showed a diffuse fibrosis with marked proliferation of biliary ductules, cholestasis, accompanied by heterophilic and lymphocytic infiltration. Although *Clostridium perfringens* (*C. perfringens*) has been suggested as the causative agent, the precise pathogenesis of the hepatic lesions remains unclear. In the present study, the relationship between cholangiohepatitis and malformation of extrahepatic bile ducts, pathomorphologic features of Ito cells in livers treated with extrahepatic bile duct ligation (BDL) and in livers of field cases were investigated in broiler chickens. The role of Ito cells in the hepatic fibrogenesis in these hepatic lesions was also discussed.

Six of the 88 abnormal livers from a slaughterhouse demonstrated biliary atresia caused by malformation of extrahepatic biliary tracts. Histologically, these livers consisted of diffuse fibrosis, diffuse proliferation of biliary ductules and distention of bile ducts,

bile plugs, multifocal aggregates of heterophils, small foci of coagulative and lytic necrosis. Although *C. perfringens* type A enterotoxin was detected in three livers by immunohistochemistry, the inflammatory reaction to these bacteria was minimal. The hepatic changes were extremely similar to those experimentally induced by extrahepatic BDL, and suggested that some cases of cholangiohepatitis are caused by biliary malformation with bile stasis.

Experimental extrahepatic BDL in broiler chickens were performed in order to clarify the pathomorphological of Ito cells in hepatic fibrosis. The treated livers were enlarged with irregular surfaces, mildly consolidated and discolored to pale green. Immunohistochemistry demonstrated that Ito cells in normal livers expressed HHF 35-specific muscle actin, vimentin, desmin, glial fibrillary acidic protein (GFAP) and cytokeratin. In treated livers, HHF 35-and desmin-positive Ito cells were frequently found in fibrotic areas and were larger in size with more extensive immunoreactivities. Ultrastructural findings suggested that Ito cells actively react