DETECTION OF EQUINE ARTICULAR CARTILAGE DEGRADATION WITH A NEW TYPE II COLLAGEN-SPECIFIC ANTI-NEOEPTIPE ANTIBODY

+*Billinghurst, R Clark (A-Bayer AG); *Buxton, E; *McGraw, M; *McIlwraith, C Wayne
+Colorado State University, Fort Collins, Colorado. Equine Orthopaedic Research Laboratory, Department of Clinical Sciences, (970) 491-4593, Fax: (970) 491-4138, rbi6@lamar.colostate.edu

RELEVANCE TO MUSCULOSKELETAL DISEASE

The destruction of the collagen fibrillar network is believed to signal the irreversible stage of articular cartilage destruction in arthritis. The ability to identify the initial collagenase-induced cleavage of cartilage-specific type II collagen would allow for the early detection and inhibition of this degradation. This study characterizes a new antibody that reacts specifically with collagenase-cleaved type II collagen fragments in the horse and demonstrates its efficacy in detecting articular cartilage degradation in this species.

INTRODUCTION

The degradation of articular cartilage, as occurs in arthritis, has been shown to involve an initial loss of aggrecan followed by the breakdown of type II collagen (1). Matrix metalloproteinases (MMPs) are involved in these processes and antibodies have been generated to detect the MMP-created termini of these two major cartilage matrix proteins. Specifically, increased cleavage of type II collagen by the mammalian collagenases MMP-1, MMP-8 and MMP-13 has been recognized in articular cartilage from the osteoarthritic joints of humans using the COL2-3/4Certer antibody (2,3). Because this antibody also reacts with cleaved type I collagen, its use to detect cartilage-specific collagen degradation products in body fluids is limited by the potential for a significant contribution of type I collagen fragments from bone, ligament, tendon and skin. We have developed an equine model that in many ways mimics osteoarthritis in humans (4) and we sought to develop an antibody that would allow us to specifically monitor type II collagen turnover in this species. By creating an immunogen containing the amino acid sequence of the collagenase-created terminus of the equine type II collagen α chain fragment, a polyclonal anti-neoeptiipe antibody could be generated for this purpose.

MATERIALS AND METHODS

A peptide was made that represented the C-terminus (neoeptiipe) of the equine type II collagen α chain fragment created by digestion with the mammalian collagenases. The peptide was conjugated to ovalbumin and used to immunize rabbits for the production of an antisem that was characterized by Western blotting for its reactivity to native and collagenase 3 (MMP-13)-cleaved equine types I, II and III collagens. The polyclonal antibody was evaluated as a true anti-neoeptiipe antibody in an ELISA using peptides with an amino acid added to or removed from the C-terminus of the immunizing peptide. Articular cartilage from the metacarpophalangeal joints of horses was cultured with recombinant interleukin-1 alpha (IL-1α), with or without a synthetic MMP inhibitor, BAY 12-9566. Type II collagen degradation was assayed using the new antibody in an inhibition ELISA. Articular cartilage from an osteoarthritic joint was compared to that of a non-arthritic equine model of osteoarthritis.

RESULTS

An affinity purified polyclonal antibody, called 234CEQ, recognized the α chain fragment created by digestion with the mammalian collagenases. This was a true anti-neoeptiipe antibody, as the removal or addition of one amino acid at the C-terminus of the immunizing peptide resulted in almost complete loss of competition for binding in an inhibition ELISA. There was a significant release from articular cartilage explants of type II collagen fragments bearing this neoeptiipe in response to IL-1 and this could be prevented with the MMP inhibitor BAY 12-9566. Articular cartilage from an osteoarthritic joint of a horse showed increased staining around chondrocyte aggregates (arrows) with the 234CEQ antibody compared to negligible staining of cartilage from a normal joint (see figure). Specificity of immunoactivity was confirmed with loss of staining on preincubation of the antibody with its peptide before its application to the cartilage sections.

CONCLUSIONS

This study has characterized an antibody and an ELISA utilizing this antibody to identify and monitor the amount of type II collagen degradation occurring in equine articular cartilage. We have shown the efficacy of this antibody in an in vitro screening system for the evaluation of novel therapeutics designed to inhibit MMP activities and specifically those of the mammalian collagenases, MMP-1, MMP-8 and MMP-13. Moreover, the 234CEQ antibody has allowed us to evaluate and localize type II collagen degradation in situ, aiding in our understanding of the changes occurring within articular cartilage at the molecular level in health and in joint disease. We are currently using the 234CEQ ELISA to determine the levels of type II collagen fragments bearing the cleavage site neoeptiipe in the sera, synovial fluids and urines of normal horses and those with various arthritides. Preliminary results indicate that this antibody will identify type II collagen degradation fragments in the body fluids of horses. The neoeptiipe that is recognized by the 234CEQ antibody is a putative biomarker for the early detection and for monitoring the progression and response to treatment of articular cartilage degradation in naturally occurring and the experimentally induced equine model of osteoarthritis.

REFERENCES