EVALUATION OF TYPE I AND II COLLAGEN BIOMARKERS FOR THE DETECTION OF JOINT PATHOLOGY IN ELEPHANTS

Emily Fyfe, MS,[1] Ellen Dierenfeld, PhD,[1,2], Trista Strauch, PhD,[1,3] Karen Wedekind, PhD[2]*

[1]Division of Animal Sciences, University of Missouri-Columbia, Columbia, MO 65211; [2]Novus International, Inc., St. Charles, MO63304; [3]Department of Fisheries & Wildlife Sciences, University of Missouri-Columbia, Columbia, MO 65211

Abstract

Degenerative joint disease (DJD) is the most prevalent musculoskeletal disease in elephants; conventional treatments for DJD in are largely palliative, including non-steroidal anti-inflammatory drugs (NSAID's) and intra-articular injections with corticosteroids or polysulfated-glycosaminoglycans (GAG's).[8,16] However, DJD is a chronic disorder, necessitating long-term treatment and there has been evidence that conventional therapies may have deleterious effects with persistent usage. Long-term use of NSAIDS may increase the risk of ulcers,[11,14,15] and both NSAIDS and corticosteroids have been shown to alter the metabolism of articular cartilage.[1,3,11] Possibly due to the current limitations of conventional therapies, DJD is the most common human and veterinary medical condition for which alternative, often nutritional, therapies are utilized.[12] Nutraceuticals, nutritional supplements intended to alter disease progression, comprise a newer class of therapies that are widely used, but whose efficacies in the treatment of DJD are largely untested.[6,8,9,16] The claimed effects are often based in anecdotal evidence or clinical trials not subject to peer review.[10] Radiographs that measure joint space narrowing are the primary diagnostic for DJD, however a significant change in joint structure can require one to two years to detect.[13] Even with more sensitive techniques, such as magnetic resonance imaging, by the time a definitive diagnosis can be established, there is often already significant joint damage.[4, 5] Additionally, current imaging technologies are not adequately sensitive to monitor the efficacy of treatments aimed at preventing or slowing DJD.[4] Alternatively, biomarkers that reflect the rate of turnover of cartilage, bone or the synovial membrane, or biomarkers that provide information about the level of oxidative stress or inflammation may offer a reliable means of evaluating the efficacy of nutraceutical therapies.[2,7,17] The first objective of this trial was to evaluate whether biomarkers for type I and type II collagen were detectable in elephant serum samples. Two commercially available enzyme-linked immunoabsorbent (ELISA) assays for the detection of the crosslinking telopeptides of type I collagen (CTX-I) and type II collagen (CTX-II) were validated for use with elephant sera. The second objective was to evaluate biomarker concentrations for correlation with joint pathology as assessed by a lameness survey (visual assessment). Sera from 41 elephants was collected via the protocols of the housing zoological institutions (n=19) and stored at -20°C until analyzed. Assays were validated using serial dilutions of samples and sample dilution curves were parallel to the standard curve for both assays. Intra-assay variability was assessed for each assay using eight replicates of high and low mixed-sample assay controls. Coefficients of variance were 5.08% and 1.51% for CTX-I and CTX-II assays, respectively. The mean serum concentration was 2.42±1.15 ng/mL for CTX-I and 19.41±43.28 pg/mL for CTX-II. Elephants assessed as having any degree of lameness tended to have lower CTX-I than sound elephants ($P<0.09$). Elephants that were assessed as markedly lame had a higher CTX-II concentration than elephants that were sound, mildly-lame or moderately-lame ($P<0.02$). There were no differences in either CTX-I or CTX-II concentrations based on whether the primary area of pathology was forelimb, hind-limb or mixed

fore- and hind limb. CTX-I concentration was higher in Asian elephants (Elephas maximus; n=7) than African elephants (Loxodonta africana; n=34; P<0.02). There was no difference in CTX-I concentration based on gender. CTX-II concentration tended to be lower in Asian elephants than African elephants (P<0.07) and higher in bulls (n=4) than cows (n=37; P<0.01). Validating available ELISA assay kits for the detection of CTX-I and CTX-II as biomarkers in elephant sera is an important step towards further investigation into early detection of joint pathology, as well as measures of therapeutic efficacy in treatment.

LITERATURE CITED

1. Brandt, K.D. 1987. Effects of nonsteroidal anti-inflammatory drugs on chondrocyte metabolism in vitro and in vivo. Am J Med 83: 29-34.
2. De Grauw, J.C., P.A. Brama, P. Wiemer, H. Brommer, C.H. van de Lest, and P.R. van Weeren. 2006. Cartilage-derived biomarkers and lipid mediators of inflammation in horses with osteochondritis dissecans of the distal intermediate ridge of the tibia. Am J Vet Res 67: 1156-1162.
3. Frisbie, D.D., C.E. Kawcak, G.M. Baxter, G.W. Trotter, B.E. Powers, E.D. Lassen, and C.W. McIlwraith. 1998. Effects of 6alpha-methylprednisolone acetate on an equine osteochondral fragment exercise model. Am J Vet Res 59: 1619-1628.
4. Garnero, P., J.C. Rousseau, and P.D. Delmas. 2000. Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. Arthritis Rheum 43: 953-968.
5. Garnero, P., E. Gineyts, S. Christgau, B. Finck, and P.D. Delmas. 2002. Association of baseline levels of urinary glucosyl-galactosyl-pyridinoline and type II collagen C-telopeptide with progression of joint destruction in patients with early rheumatoid arthritis. Arthritis Rheum 46: 21-30.
6. Hanson RR, Brawner WR, Blaik MA, et al. 2001. Oral treatment with a nutraceutical (Cosequin) for ameliorating signs of navicular syndrome in horses. Vet Ther 2:148-59.
7. Jansen, N.W., G. Roosendaal, B. Lundin, L. Heijnen, E. Mauser-Bunschoten, J.W. Bijlsma, M. Theobald, and F.P. Lafeber. 2009. The combination of the biomarkers urinary C-terminal telopeptide of type II collagen, serum cartilage oligomeric matrix protein, and serum chondroitin sulfate 846 reflects cartilage damage in hemophilic arthropathy. Arthritis Rheum 60: 290-298.
8. Kawcak, C.E., D.D. Frisbie, C.W. McIlwraith, N.M. Werpy, and R.D. Park. 2007. Evaluation of avocado and soybean unsaponifiable extracts for treatment of horses with experimentally induced osteoarthritis. Am J Vet Res 68: 598-604.
9. Laverty S, Sandy JD, Celeste C, et al. 2005. Synovial fluid levels and serum pharmacokinetics in a large animal model following treatment with oral glucosamine at clinically relevant doses. Arth Rheum 52:181-191.
10. McIlwraith, C.W. 2004. Liscensed medications, "generic" medication, compounding, and nutraceuticals-what has been scientifically validated, where do we encounter scientific mistruth, and where are we legally? . In: Proceedings of the 50th Annual Convention of the American Association of Equine Practitioners, Lexington, KY. 459-475.
11. Moskowitz, R.W. 1996. The appropriate use of NSAIDs in arthritic conditions. Am J Orthop 25: 4-6.
12. Resch, K.L., S. Hill, and E. Ernst. 1997. Use of complementary therapies by individuals with 'arthritis'. Clin Rheumatol 16: 391-395.

13. Sandell, L.J., and T. Aigner. 2001. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Res 3: 107-113.
14. Snow, D.H., T.A. Douglas, H. Thompson, J.J. Parkins, and P.H. Holmes. 1981. Phenylbutazone toxicosis in equidae: a biochemical and pathophysiological study. Am J Vet Res 42: 1754-1759.
15. Traub-Dargatz, J.L., J.J. Bertone, D.H. Gould, R.H. Wrigley, M.G. Weiser, and S.D. Forney. 1988. Chronic flunixin meglumine therapy in foals. Am J Vet Res 49: 7-12.
16. Troy, N.T. 2005. The Use of Nutraceuticals for Osteoarthritis in Horses. The Veterinary clinics of North America. Equine practice 21: 575-597.
17. Williams, F.M., and T.D. Spector. 2008. Biomarkers in osteoarthritis. Arthritis Res Ther 10: 101.