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BEYOND THE X-RAY: THE LATEST METHODS TO DETECT AND PREDICT SKELETAL DAMAGE

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Introduction

While osteochondral fragmentation, fractures, subchondral bone disease, and osteoarthritis are common in the horse, diagnosis of these diseases usually occurs only after the disease has become established. The detection of early or subtle disease in the past has been poor, but the situation is improving. Clinical examination and radiographic imaging are still the most commonly used techniques for diagnosis of osteochondral disease, yet osteochondral damage seen during arthroscopic surgery is usually more severe than that seen on radiographs. It is the author's subjective opinion that there is commonly a good correlation between the severity of clinical symptoms (principally lameness and synovial effusion) and the amount of damage or disease found at arthroscopy of joints. However, it has been reported in humans that, while the most common complaint of the patient with osteoarthritis (OA) is pain, only about half of the patients with radiographic OA have symptoms (Hochberg et al., 1989). The reason that half the patients with radiographic OA have pain is not always clear since only some of the causes of pain have been researched (Altman and Dean, 1989). It is recognized that there is no "diagnostic test" for OA in man (Altman, 1997), but focus on MRI and biomarkers has occurred in recent years.

Human clinical trials are now very specific about the recording of outcome measures. Outcome variables in OA clinical trials need to be selected on the basis of the therapeutic objective and are a critical part of assessing the results of medication. In a workshop of the World Health Organization and the American Academy for Orthopedic Surgeons, the methods to assess progression of OA of the hip and knee were reviewed (Dieppe et al., 1994). In addition, the European Group for the Respect of Ethics and Excellence in Science (GREES) has made recommendations on methods for registration of drugs for OA (1996). We are equally in need of objective outcome parameters in assessing the results of various treatments for musculoskeletal disease in general and joint disease in particular in the horse.

It has been proposed that to completely characterize joint disease in the horse, the following measurements are necessary: 1) mechanical inputs into the joint; 2) tissue architecture and geometry; 3) tissue matrix properties, including measurement of material and biochemical matrix properties; and 4) the level of inflammation within the joint (Kawcak, 2001). The current state of diagnostic capabilities for horses will

be presented here. Some of these are already being used by clinicians, some are ready to be used, and some are futuristic.

Measurement of Mechanical Inputs

CLINICAL EXAMINATION

Assessment of joint effusion, range of motion, joint capsule thickening, and pain with flexion are currently used and subjectively graded by veterinarians doing lameness examinations. The lameness grading guidelines set up by the American Association of Equine Practitioners are used frequently (AAEP, 1991). While flexion tests are commonly used in humans, the reliability of such tests is controversial. Confounding factors make this objective evaluation of an individual for pain difficult. The principal ones are differences in observer scores and differences in a particular subject's tolerance to pain. The same is true for a horse. Motion analysis has been employed as a research tool. The characteristics of limb movement and force can be determined. Abnormalities in these parameters can be characterized in patients with disease (Craik and Otis, 1995). As an example, it has been found in humans that impulsive loading often leads to osteoarthritis (Radin and Rose, 1986). Because data analysis involves sophisticated, expensive equipment and is often labor intensive, most gait analysis in veterinary medicine occurs in the research field.

Motion analysis systems that combine data from force plates, EMG analysis and muscle forces, and kinematics can provide sensitive information about an individual's movement (Radin and Rose, 1986). These systems have been extensively studied in humans and are used clinically to evaluate an individual's gait. Limb use and muscle forces play a large role in joint loading (Bassey et al., 1997), and recent work from our laboratory in the horse has shown this. For instance, in measuring contact forces across the carpus at the trot, the peak ground reaction force is 1,350 pounds, whereas the peak muscle forces are 2,700 pounds, leading to a total joint force of 4,050 pounds. In other words, muscle forces are two times ground forces (Brown et al., 2003a; Brown et al., 2003b). Diagnostic techniques that describe kinematics and muscle forces are research tools that allow clinicians to identify those individuals with potential problems related to movement.

We have also evaluated the use of thin-film sensor systems to evaluate limb loading in horses. The system was attractive because a sensor could be attached to the bottom of a horse's hoof to measure force distribution throughout the sole surface (Judy et al., 2001), or it could be used like a force plate for jogging horses across. Evaluation of the force plate system for accuracy and durability has shown it to be inadequate.[a] Preliminary results indicate, however, that using an "in-shoe" system for the sensor film deploys results similar to the force plate.[b]

COMPUTER MODELS

Computer models of joint loading have been studied in both humans and animals. Modeling is the computer-based mathematical representation of the skeleton, ligaments, and muscles used to calculate forces in muscles and joints. The principle is to develop the model based on kinematic parameters and compare that model to those developed from imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Muscle, tendon, ligament, and ground reaction forces in tissue properties can be inserted into the model. Once developed, imaging-based modeling can be performed so that subtle changes in joint geometry and loading can be detected with the ultimate goal to develop long-term models in which data can be continually added. The clinical goal is to develop patient-specific models in which abnormalities in loading and tissue response can be detected (Pandy et al., 1998).

Researchers in the Equine Orthopedic Research Laboratory at Colorado State University (CSU), in collaboration with the Orthopedic Research Laboratory at Columbia University and Steadman Hawkins Sports Medicine Foundation, have performed a kinematic and MRI study to develop a model of the equine carpus. This study has determined the center of force of the joint surfaces in the carpus, and these data have been correlated with those obtained from MRI scans. At the moment this is certainly a research tool, but ultimately we hope the subtle irregularities in joint loading can be determined in clinical patients using MRI and CT.

Measurement of Tissue Architecture and Geometry

RADIOGRAPHY

Radiography is still the most widely used imaging technique for the diagnosis of osteochondral disease, but it is an insensitive method of diagnosis. Articular cartilage cannot be viewed radiographically except when there is extensive loss and decreased joint space, and 30-40% change in bone mineral density is required before bone changes can be appreciated (Greenfield, 1986). In addition, multiple images are required for evaluation of a three-dimensional structure. Disease is often recognized after significant damage has occurred. This lack of sensitivity can prevent early and accurate diagnosis. Measuring joint space is fraught with error (Adams and Wallace, 1991). The significance of osteophytes is frequently unrelated to intra-articular pathological change and considerable change in bone density is necessary to identify sclerosis and erosion. In a study correlating radiographic and histologic changes in the tarsi of horses, Laverty and coworkers found that radiographs were insensitive for detecting subchondral bone sclerosis and erosion when compared to histology (1991). It has also been pointed out that superimposition of osteophytes may appear as sclerosis (Widmar and Blevins, 1994).

COMPUTED TOMOGRAPHY

Computed tomography (CT) has had increasing use in the horse, both as a research tool and a clinical tool. Benefits of CT are visualization of the area of interest in three dimensions (which alleviates superimposition) and the ability to determine density patterns.

Density patterns of bone can be determined by three-dimensional modeling of CT images (computed tomography osteoabsorptiometry or CTO). CTO allows three-dimensional evaluation of the joint in any plane. Hounsfield units, which are the CT measure of bone density, are determined and coordinated into ranges and then the ranges of density are represented by colors. This color map is then superimposed over a three-dimensional image of the joint surface to show a representation of the relative subchondral density (Figure 1).

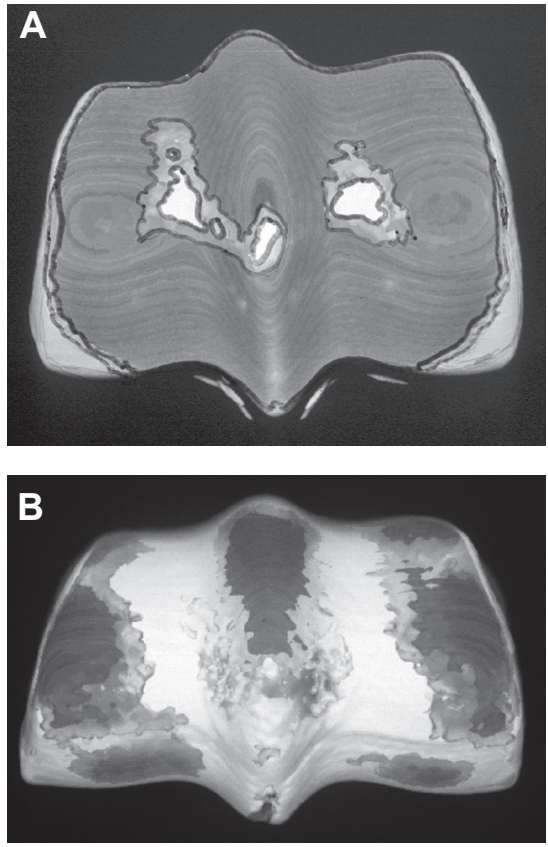

Figure 1. Computed tomography osteoabsorptiometry (CTO), a three-dimensional evaluation of the surface of the third metacarpal condyles of a horse exercised on a treadmill (A) and a hand-walked horse (B). Notice the increased density of subchondral bone (black areas) in the treadmill-exercised horse compared with the hand-walked horse (reprinted from Kawcak et al., 2000. Amer. J. Vet. Res.).

The use of a density phantom has allowed for objective measures of density to be determined. Since it has been shown that stress distribution within an osteochondral section is related to the density pattern, it can be concluded that the subchondral density pattern is the representation of the loading history of the joint (Muller-Gerbl et al., 1989). Considerable work has been done in our laboratory by Kawcak. Initially the subchondral density patterns of bones in equine carpal and metacarpophalangeal joints were established. Since that time the effects of exercise in young horses where exercise was commenced in foals at three weeks have been followed and compared to those in pasture-reared horses. In addition, we have evaluated the changes in bone density patterns with age.[c]

Riggs and coworkers identified substantial density gradients between the denser subchondral bone of the condyles and the subchondral bone of the sagittal groove in the distal MCIII and MTIII with a view to explaining the etiology of distal condylar fractures (1999). These density gradients were shown to equate to anatomical differences in loading intensity and locomotion, and it was hypothesized that such difference in bone density results in stress concentration at the palmar/plantar aspect of the condylar groove, which may be predisposed to fracture (Riggs et al., 1999a). In a companion paper, linear defects in mineralized articular cartilage and subchondral bone were found in the palmar/plantar aspects of the condylar groove, adjacent to the sagittal ridge (Riggs et al., 1999b). These were closely related to the pattern of densification of subchondral bone and were associated with intense focal remodeling of the immediate subjacent bone. Parasagittal fractures of the condyles originated in similar defects. This work and subsequent examination of CTs in our laboratory have demonstrated a potential to diagnose incipient condylar fractures in the racehorse.

MRI

Results from human studies have shown that MRI is a sensitive and specific imaging tool for examination of hard and soft tissues in joints and that it is as good as, if not better than, arthroscopy for detecting subchondral lesions (Reeve et al., 1992). MRI is the best measure of articular geometry, and more recently an ability to quantify articular cartilage matrix properties using contrast enhancement has been demonstrated (Bashir et al., 1997). Postmortem MRI, as well as other imaging modalities including clinical examination, radiographs, nuclear scintrigraphy, and arthroscopy, was used to evaluate an osteoarthritic metacarpophalangeal joint in a horse (Martinelli et al., 1996). Kawcak and coworkers have also used this technique to evaluate the effects of exercise on subchondral bone of horses and found that it could image osteochondral damage, including small fragments (Figure 2) (Kawcak et al., 2001).

More recently there have been reports of the clinical use of MRI (high-field strength) in anesthetized horses to diagnose specific changes in the distal limb and a paper on the use of a low-field strength standing MRI to image the distal limb has been reported (Dyson et al., 2005; Mair et al., 2005).

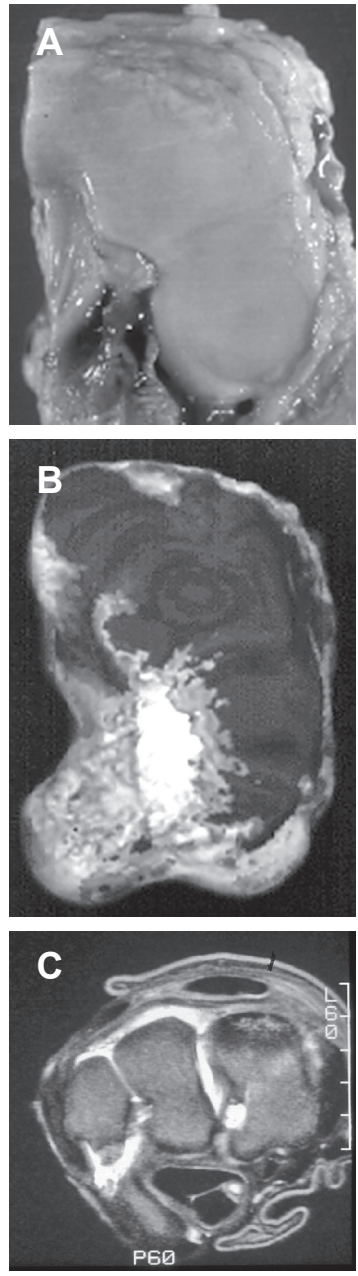

Figure 2. Imaging of an osteochondral fragment on the distal aspect of the radial carpal bone: (A) gross photographic view; (B) a CTO image; and (C) an MR image of the distal aspect of the radial carpal bone showing the fragment (reproduced with permission from C.E. Kawcak, 2001. Proc. Amer. Assoc. Equine Practnr.).

We have had a high-field strength MRI at CSU for a year, and it is being used effectively on clinical patients to diagnose problems from the tarsus and carpus down. Changes in the joint capsule and ligaments associated with joints can be diagnosed equally well as those in articular cartilage and bone (Figure 3).

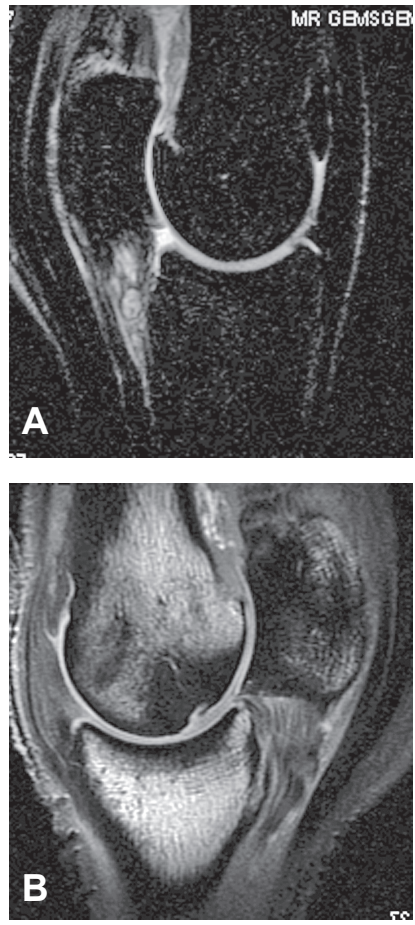

Figure 3. (A) MRI of tearing of the oblique distal sesamoidean ligament (STIR sequence). (B) MRI of bone edema and sclerosis with defect in distal metacarpus (T1-weighted spin echo sequence). Both images are from racehorses (courtesy of Dr. N. Werpy).

ULTRASONOGRAPHIC EXAMINATION

Ultrasonographic examination of joints was pioneered by Denoix (1996). The technique can be used to evaluate soft tissues associated with the joint, including collateral ligaments, joint capsule, other associated ligaments, and menisci (Marks et al., 1992). The use of ultrasonography to image the medial palmar intercarpal ligament in the carpus has also recently been described (Driver et al., 2004).

Measurement of Tissue Matrix Properties, Including Measurement of Material and Biochemical Matrix Properties

NUCLEAR SCINTIGRAPHY

Nuclear scintigraphy has been extremely helpful in detecting cortical bone disease and, in particular, stress fractures in horses. Its most significant use, in my opinion, has been in detecting stress fractures of the pelvis, tibia, femur, and humerus prior to their becoming complete fractures. A nuclear scintigraphic image shows the physiologic distribution of radioisotope throughout the bone and therefore is more sensitive than radiographs in detecting early osteoarthritis in human knees (McCrae et al., 1992). In humans, nuclear scintigraphy has been the best early predictor of joint space narrowing in knees, and in some cases has been more sensitive than arthroscopy and MRI for detecting early and subtle subchondral bone pathology (Marks et al., 1992; Dieppe et al., 1993). One problem, however, is the inability of nuclear scintigraphy to distinguish stress response due to subchondral bone adaptation from osteochondral damage. Osteochondral fragments show up as discrete focal areas of increased radioisotope uptake, but any remodeling change due to stress will also show increased uptake of radioisotope (Chambers et al., 1995; Parks et al., 1996). Because of these, mild to moderate increases in uptake of radioisotope in the joints of horses, especially young exercising horses, can lead to confusion. However, scintigraphy can be used as a screening tool, but it needs to be recognized that, while sensitive, it is not sensitive enough to demonstrate a specific anatomical problem.

More objective means of assessment have been used to eliminate some of the subjectivity with nuclear scintigraphy. Using computer programs, areas of particular interest can be highlighted, the counts per pixel determined for that area, and normalized to the counts per pixel for a reference area within the same limb (Wittbjer et al., 1982). This is of particular benefit because the distribution of radioisotope within an area varies between animals and between different regions within the same animal. If we outline an area of interest such as the distal condyles of the third metacarpus, and then normalize the count to a reference area such as the cortical area of the first phalanx, it is possible to eliminate the influence of individual horse uptake in assessing this area (Figure 4).

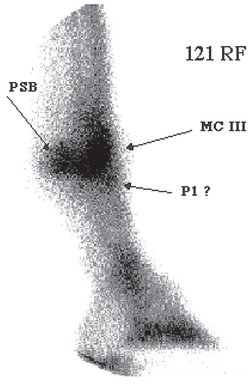

Figure 4. Nuclear scintigraphic view of a metacarpophalangeal joint of a healthy horse. PSB, proximal sesamoid bone; MCIII, third metacarpal condyle; P1?, presumed proximal phalanx (reproduced with permission from Kawcak et al. 2000. Amer. J. Vet. Res.).

Care needs to be taken to ensure the reference area is normal and has no increased uptake compared to the surrounding bone. This technique takes into account the regional limb response to exercise and therefore potentially reduces the effect of exercise-induced increases.

Material properties of subchondral tissues can be inferred from the CT examination. The densities of subchondral and cortical bone have shown to be proportional to their strengths; therefore, a measure of bone density can give the clinical impression of bone strength (Wachter et al., 2001). This is commonly used in an outpatient setting for humans in which peripheral quantitative CT (pQCT) is used for diagnosis and to monitor therapy for osteoporosis. However, unlike cortical bone, there is a maximum density at which subchondral bone can be damaging to articular cartilage (Radin and Rose, 1986). From this it can be insinuated that there exists an appropriate density range at which subchondral bone must be maintained in order to avoid joint damage, a range which we have not yet determined (Kawcak, 2001).

Histologic properties of osteochondral tissues can be assessed using optical coherence tomography, and this has been likened by Kawcak to an in vivo form of biopsy (Herrmann et al., 1999; Kawcak, 2001). In humans, optical coherence tomography has shown a fairly good correlation between images and histologic change (Herrmann et al., 1999).

SYNOVIAL AND SERUM BIOMARKERS

Conventional synovial fluid analysis will not provide a specific diagnosis, but it will give an indication of degree of synovitis and metabolic derangement within the joint. It will not define the degree of articular cartilage damage, but merely the degree of synovitis. Previous attempts at techniques such as synovial sediment analysis have also not solved this problem. Over the past 10-15 years, researchers have developed biochemical and immunologic biomarkers to quantitate breakdown products of the articular cartilage, and the use of this technique in the horse has been the subject of reviews (Ray et al., 1996; McIlwraith et al., 2001).

The terms biomarker, biochemical marker, and molecular marker have all been used to describe either direct or indirect indicators of musculoskeletal turnover (McIlwraith et al., 2001). These markers are often molecules that are normal products and by-products of the metabolic process occurring within the musculoskeletal system. Disease alterations occur between the anabolic and catabolic processes within the skeletal tissues, and consequently, concentration of biomarkers may increase or decrease. In joint disease these molecules can be released into the synovial fluid when the source is articular cartilage, menisci, ligament, or synovial membrane. If the underlying subchondral bone is involved, molecules from osseous tissue will usually be delivered into the bloodstream. Biomarkers potentially can be used to 1) clarify pathobiological processes in the joint; 2) differentiate diagnostically between affected and nonaffected joints and distinguish the degree of degradation in articular cartilage; and 3) monitor the response to therapy.

Direct biomarkers originate principally from cartilaginous structures and provide specific information about alterations in cartilage matrix, anabolism, or catabolism (Thonar et al., 1999). On the other hand, indirect biomarkers are not derived principally from cartilage but have the potential to influence the metabolism of chondrocytes or the integrity of the matrix. These include proteolytic enzymes and their inhibitors, growth factors, pro-inflammatory cytokines, and other molecules from noncartilaginous sources that can provide information, including MMPs, aggrecanase, TIMP, IGF-I, IL-1, IL-6, TNFα, HA, and C-reactive protein (CRP). Indirect markers used in the horse have been recently reviewed (McIlwraith, 2005).

Individual Direct Biomarkers of Cartilage Metabolism

BIOMARKERS OF ANABOLIC PROCESSES

The carboxypropeptide of type II collagen (CPII) is a useful measure of type II collagen synthesis. Although CPII concentrations were not significantly higher in synovial fluid samples of joints with osteochondral fragmentation, their levels were significantly higher in the serum of horses with osteochondral fragmentation (Frisbie et al., 1999). In the same study with horses, another synthetic marker, chondroitin sulfate 846 (CS-846), was significantly higher in the synovial fluid of joints with osteochondral fragmentation compared to control joints, and serum levels were also significantly higher (Frisbie et al., 1999). CS-846 and CPII concentrations were not linearly related to greater fragmentation but were significantly higher with grades I and II. Discriminate analysis using a combination of serum CS-846 and CPII concentrations allowed for 79% of horses to be correctly classified as having osteochondral damage.

BIOMARKERS OF CATABOLIC PROCESSES

Measuring the degradation of type II collagen with biomarkers has proven to be a benefit in monitoring OA as well as OCD in the horse. Antibodies have been developed to identify type II collagen fragments that have been cleaved and/or denatured, exposing previously inaccessible regions (neo-epitopes) of the molecule. Using these antibodies, significant elevations in levels of degraded type II collagen have been demonstrated in synovial fluid and serum samples from horses, dogs, and rabbits with experimental OA (Billinghurst et al., 1997). Initially the Col-2-3/4$_{short}$ immunoassay for detecting collagenase-cleaved collagen fragments (able to detect both type I and II collagen degradation) was developed. This assay had been used in the author's laboratory for monitoring collagenase-induced collagen degradation and to measure the inhibitory effect of a synthetic MMP inhibitor on IL-1-induced degradation of equine articular cartilage explants (Billinghurst et al., 1999). More recently a collagen degradation immunoassay that is specific for equine type II collagen degradation was developed (Billinghurst et al., 2001). The antibody in this assay is designated as 234CEQ.

In a recent study of skeletal markers in osteochondrosis in foals, a combination of significantly higher serum levels of CPII, higher levels of Col-2-3/4$_{short}$ and lower levels of 234CEQ correlated with high osteochondrosis (OC) scores (radiographically) (Billinghurst et al., 2004). This study suggests that there is increased collagen turnover in OC, and by measuring the serum levels of specific biomarkers of collagen metabolism, it is possible to identify horses with OC (Billinghurst et al., 2004). An earlier study in cases of OC found that there were significantly higher levels of CPII and lower levels of CS-846 and KS epitopes in synovial fluids of affected compared to normal joints (Laverty et al., 2000).

Other biomarkers that have been measured in the horse include keratin sulfate (KS) and cartilage oligomeric protein (COMP), but up until now these have proven less useful. On the other hand, the development of monoclonal antibodies that distinguish the two different sites of aggrecan degradation can help identify which is the most responsible for aggrecan degradation in the horse (Caterson et al., 2000). At present it appears that aggrecanase is much more important that stromelysin in this degradation process.

Individual Direct Biomarkers of Bone Metabolism

BIOMARKERS OF ANABOLIC PROCESSES

During normal type I collagen synthesis, as with type II collagen, cleavage of carboxy and amino terminal propeptides (PICP and PINP respectively) of the procollagen molecule occurs and these cleaved propeptide fragments can be exploited as markers reflective of bone formation. In a preliminary study PICP was shown to have potential value as a molecular marker for monitoring changes in matrix turnover following tendon injury (Jackson et al., 2003), and increases in PICP with age and exercise have been demonstrated (Price et al., 1995; Price et al., 2001).

Osteocalcin is a small, noncollagenous protein associated with bone assembly and turnover, and levels in the horse appear to vary with age or administration of corticosteroids, as well as general anesthesia. In a study in our laboratory where various serum markers were used to differentiate changes with exercise from pathologic change in joints, concentrations of osteocalcin as well as CS-846 provided the best correlation to the modified Mankin score (r2=0.72) and clinical degree of pain (r2=0.70) using multivariate linear regression (stepwise model selection) (Frisbie et al., 2003).

Bone-specific alkaline phosphatase (BAP) is expressed at high levels in the cell surface in the bone-forming osteoblasts. In a study with treadmill exercise in young horses, serum BAP levels were not different between exercise and control groups, although previously there had been a suggestion that there was a correlation between levels of BAP and the amount of arthroscopically defined joint damage (Fuller et al., 2001).

BIOMARKERS OF CATABOLIC PROCESSES

The release of a fragment of the type I collagen nonhelical telopeptide (ICTP), which includes the collagen cross-linking region, has been evaluated as a marker of bone resorption in humans (Cortet et al., 1997; Garnero et al., 1999). Levels of ICTP in the horse have not been of value in detecting pathological processes (Price et al., 1995; Jackson et al., 2003).

A relatively new set of antibodies recognizing type I collagen C-telopeptides (CTX) has proven to be useful markers of specific bone resorption based on clinical data from cases of human joint disease (Bonde et al., 1997). In the same study from our laboratory evaluating the ability of serum markers to differentiate exercise from pathology and correlate biomarkers to clinical parameters of pain in an osteoarthritic model, CTX was less useful than CS-846, CPII and GAG biomarker levels in predicting if serum was from either a control, exercised, or an osteoarthritic horse (Frisbie et al., 2003). Other work in our laboratory has identified CPII and CTX-1 as potential serum indicators of the exercise effects on the developing skeletal system in young horses. There were higher serum levels of CTX-1 and lower levels of CPII in trained foals compared to other groups, but these differences later disappeared during an additional six months of identical exercise (Billinghurst et al., 2003).

One of the principal aims of biomarker research is to diagnose early subchondral bone disease and thereby potentially predict fracture. This was the basis of a study funded by the Grayson-Jockey Club Foundation and carried out with racing Thoroughbreds in southern California.

Gene Chip Microarray

Gene chip microarray is the latest advance in biomarkers and represents a molecular approach to defining a disease process. The principle is to have an array of a large number of gene sequences (cDNAs) on a computer chip. The entire human genome is currently available on a computer chip (Affymetrics) and the same company, in corroboration with the Australian company Genetraks®, has produced an equine gene chip containing over 3,000 sequences. The production of this chip facilitates the simultaneous relative quantitation of multiple mRNAs and allows for comprehensive assessment of expression levels.

In recent work from our laboratory (Frisbie et al., unpublished data), the potential usefulness of gene chip microarray as a diagnostic tool in osteoarthritis has been explored. Blood samples were taken during the development of experimental OA (using the carpal chip fragment-exercise model) in the horse, and we were able to identify significant upregulation of 18 different genes in the osteoarthritis group compared to the controls. This change in gene expression started very early in the development of the osteoarthritic disease process. It is envisioned that this ability will be combined with conventional immunologic biomarkers (previously discussed) to provide a diagnostic platform for osteoarthritis as well as other diseases.

Summary

We are progressing towards the ability to diagnose cartilage and bone disease in an individual with a single sample using biomarkers. We are not there yet, but we are at the stage where we can monitor disease process with regular sampling.

Footnotes

[a] Perino, V. 2003. M.S. thesis, Colorado State University, Ft. Collins, CO.
[b] Perino, V., C.E. Kawcak, D.D. Frisbie, et al. 2005. Unpublished data.
[c] Shearin, M. 2005. Ph.D. dissertation; Colorado State University, Ft. Collins, CO.

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