

Diagnostic Importance of Deoxypyridinoline and Osteocalcine in Equine Osteoarthritis

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Abstract

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In this study, deoxypyridinoline (Dpd) and osteocalcine (OC), which has been considered as a diagnostic and prognostic marker for joint disease in man and dog, was measured in urine from horses with osteoarthritis (OA). Serum and urinary assays of bone markers provide a noninvasive alternative to bone biopsy in the study of bone metabolism in horses.

No significant differences in serum osteocalcine were found in either group. However, significant differences were found in deoxypyridinoline/creatinine ratio between normal and OA urine. They may be useful in the monitoring of therapy and prognosis in horses with osteoarthritis. The measurement of collagen crosslink, deoxypyridinoline is comparatively more specific to monitor bone resorption. This article reviews the use of different bone markers in veterinary medicine and the possibilities for diagnosing and preventing bone diseases of horses.

Horses, osteoarthritis, osteocalcine, deoxypyridinoline, degenerative joint disease.

Degenerative joint disease (DJD) may be considered as a group of disorders characterized by a common endstage: progressive deterioration of the articular cartilage accompanied by changes in the bone and soft tissues of the joint. There have been various interpretations of DJD in the horse. There has been a lack of correlation between pathologic changes has not been well defined (McIlwraith 1982, 1987).

Clinical significance of lesions that extend into subchondral bone is not disputed; several investigators have detected a decrease in one or more of the glycosaminoglycans in osteoarthritic cartilage (Poole 1994; Arican et al. 1994a, 1994b; Fuller et al. 2001) and this decrease was in direct proportion to the severity of the disease morphologically.

Studies of cartilage and bone biochemistry have been important in studying the mechanisms of OA and the search for a marker of joint damage resulting from osteoarthritis. The aim of molecular marker research is to detect OA changes early and non-invasively, before irreversible disease processes have occurred. Potential biological markers glycosaminoglycans, keratan sulphate, chondroitin sulphate, hyaluronan for equine osteoarthritis have been recently studied (Fuller et al. 2001). Most of the potential markers have been of inflammatory processes, cartilage destruction (Poole and Dieppe 1994) and bone erosion (Poole 1994; Garnero and Delmas 1997).

A number of commercial and urine assays have been developed to provide non-invasive monitoring of bone formation and resorption in humans (Fujimoto et al. 1978; Eyre et al. 1984). The ideal marker of cartilage degradation is one that is not found in normal cartilage or in any other tissue. Deoxypyridinoline (Dpd) and pyridinoline are used in human medicine for diagnosis and evaluation of bone diseases and in predicting the occurrence of

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fractures and rates of bone loss (Eastell et al. 1997). The carboxy terminal telopeptide of type I collagen, which has been used in several animal species, is also a promising bone marker (Arıcan et al. 1995; Ladlow et al. 2002).

Pyridinoline is the major cross-link of collagen and is prevalent in cartilage and several other tissues, whereas Dpd is primarily located in bone (Fujimoto et al. 1978; Ogawa et al. 1982; Eyre et al. 1984; Robins and Duncan 1987). Deoxypyridinoline is found predominantly in type I of bone (Robins 1983; Eyre et al. 1984). Dpd components have been used to assess collagen degradation rates by quantitative measurements in urine, based on either immunoassay (Robins 1983) or high performance liquid chromatography (HPLC) (Eyre et al. 1984; Black et al. 1988). More recently, these techniques have been applied to assess collagen degradation in arthritic diseases (Seibel et al. 1989) and in a range of metabolic diseases (Robins et al. 1986; Uebelhart et al. 1990; Uebelhart et al. 1991). Deoxypyridinoline, primarily a bone cross-link, is found in increased amount in urine in metabolic bone disorders such as osteoporosis (McLaren et al. 1992). Urinary deoxypyridinoline is elevated in animals and humans with OA, presumably as a result of bone breakdown or repair. In patients with OA it is due to loss of collagen matrix primarily from the articular cartilage and bone of the joint. Deoxypyridinoline is useful in the monitoring of therapy in animals with OA (Arıcan et al. 1995).

Osteocalcine is one of the most abundant non-collagenous proteins of the bone, constituting up to 3% of total bone protein. Osteocalcine is thought to function in bone tissue formation as it is produced by osteoblasts. The anabolic activity of osteoblasts may be determined by measuring serum concentrations of osteocalcine (Jaffe et al. 1994; Arıcan et al. 1995; Grafenau et al. 2000; Lepage et al. 2001).

The aim of this study was to determine the concentrations of serum osteocalcine and deoxypyridinoline cross-link excretion in the urine of normal horses and horses with naturally occurring OA, with a view to evaluating the potential usefulness of cross-link measurement for diagnosis and prognosis.

Materials and Methods

Sample collection

Urine and blood samples were obtained from horses under sterile conditions. After a conventional health measures, all procedures have been performed in accordance with national local animal welfare legislation. Measurements of deoxypyridinoline were performed on a 2-hour fasting morning urine samples. Urine samples were stored in aliquots at -20°C in dark until assayed.

The diagnoses of OA (12 horses sampled) were based on history, following clinical examination (severe trauma, joint instability), intra-articular anesthesia and radiographic assessment (Table 1). The clinically affected joint was defined as that principally responsible for presenting lameness. Most cases involved the carpal, hock and fetlock joints. The majority of OA cases in this study were from 'secondary' osteoarthritic cases, i.e. developed secondarily to another primary joint problem.

Ten animals with normal joints were selected from clinical cases presented at the horse riding club with no history of locomotor disease; the absence of joint disease was confirmed by clinical examination and radiography (Table 1).

Osteocalcine assay

Plasma concentrations of osteocalcine was evaluated by the optimized standard method of immunometric assay using the Immulite automatic analyser (DPC, Los Angeles). Range was between 3.1-13.7 for osteocalcine levels. High and low equine samples were included in each assay to ensure reproducibility.

Deoxypyridinoline assay

Urine concentrations of deoxypyridinoline assay was evaluated by the optimized standard method of competitive immunoassay using the Immulite automatic analyser (DPC, Los Angeles). High and low equine samples were included in each assay to ensure reproducibility.

Creatinine assay

The methods most commonly used for creatinine determination are based on the Jaffe reaction (Butler 1975), the conversion to a yellow-orange compound with picric acid in the presence of alkaline solution. The concentration of this compound indicates the creatinine concentration in the sample. The optical density measured in a spectrophotometer (Olympus Diagnostica, Hamburg).

Table 1
Breed and age distribution in horses with arthritis and normal horses

Arthritis	Breed	Age (years)
1	Thoroughbred	11
2	Thoroughbred	10
3	Thoroughbred	11
4	Thoroughbred	10
5	Arabian	11
6	French Saddle	18
7	Haflings	7
8	Thoroughbred	7
9	Thoroughbred	4
10	Thoroughbred	4
11	Thoroughbred	5
12	Arabian	8
Control	Breed	Age(years)
1	Thoroughbred	11
2	Arabian	5
3	Pony (Shetland)	8
4	Arabian	4
5	Thoroughbred	5
6	Thoroughbred	3
7	Thoroughbred	4
8	French Saddle	4
9	Akalteke	5
10	Pony (Shetland)	8

Interpretation of results

To correct for variations in urinary flow, deoxyipyridinoline results should be normalized to the urinary creatinine concentration, and expressed as nanomoles deoxyipyridinoline, per liter divided by milimoles creatinine per liter (nM deoxyipyridinoline/mM creatinine).

Conversion Factor

Creatinine $\text{mg}\cdot\text{dL}^{-1} \times 0.088 \text{ mmol/L creatinine}$

Statistical Analysis

Patient group data were statistically compared by the Mann Whitney U test, plus the corresponding one standard deviations were used as the cut-off point for positivity.

Correlation coefficients were determined by simple linear regression using of statistics packet programme (SPSS, Minitab).

Results

Osteocalcine in sera

Concentrations of OC in sera are summarized in Table 2. There were non-significant differences in the osteocalcine concentrations between normal and OA sera.

Table 2
Osteocalcine (OC) concentration in sera and deoxyipyridinoline /creatinine (Dpd) concentration in urine from horses with osteoarthritis and normal (controls) (Median -Range)

Group of patients	number	Median (Max-Min) concentration Deoxyipyridinoline (Dpd) (nmol/mmol creatinine)	Median (Max-Min) concentration Osteocalcine ($\text{ng}\cdot\text{mL}^{-1}$)
Control	10	5.5 (9.2-3.5)	<1.0
Osteoarthritis	12	9.3 (23.4-5.2)	1.0

Deoxyypyridinoline/creatinine in urine

The deoxyypyridinoline/creatinine ratios in urine are summarized in Table 1.

There were significant differences in the deoxyypyridinoline/creatinine ratios between normal and OA urine (Fig. 1).

Correlations of Dpd and OC

There were no correlations between Dpd levels or OC levels and age of horses.

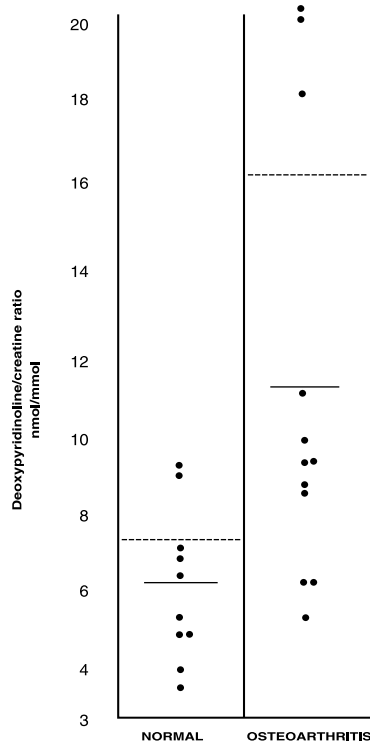
Discussion

In the present study, we have measured cross-link (deoxyypyridinoline) concentrations in horse urine. The data for deoxyypyridinoline were expressed relative to creatinine in order to correct for variations in urine volume. There were significant differences between deoxyypyridinoline in normal and OA equine urine samples. The differences between some of these samples are most likely to be explained by the degree of joint, and hence cartilage damage. The overlap between groups, however, made further analysis and interpretation difficult. There was no correlation between radiographic evidence of cartilage/bone damage and deoxyypyridinoline/creatinine ratios (Black et al. 1999).

There was no significant relationship between deoxyypyridinoline/creatinine ratios and age of the horse by linear analysis. The horses studied here covered a range of breeds and sizes and we were not able to show any clustering to suggest predisposition in particular breeds. However, this issue should be considered in future studies. Our studies only included 22 horses for which we had urine samples. For breed studies, a larger number of cases would be required before firm conclusions could be drawn.

It has been shown that pyridinoline and deoxyypyridinoline are elevated in some human patients with RA, OA and also in dogs (Robins et al. 1986; Seibel et al. 1989; Arican et al. 1995; Ladlow et al. 2002). It is well known that joint destruction in joint diseases is associated with two types of bone loss: periarticular bone loss, which is an early radiological finding in joint diseases, associated with the local inflammatory process, and generalised bone loss.

Fig. 1. Deoxyypyridinoline/creatinine ratios in urine from horses with osteoarthritis and normal (controls) horses. Each point represents one horse and the bar represents the mean for each group. The line showing the cut-off for positivity is derived from the mean plus one standard deviations.



McLaren et al. (1992) showed that the excretion of pyridinoline and deoxyypyridinoline was significantly higher in people with fractures and with osteoporosis. The fact that there were differences in the molar deoxyypyridinoline between the patient groups supports the view that the major part of the cross-links in urine are derived from bone. On the other hand, Pyd and Dpd are formed from the breakdown of mainly type II collagen, and have been shown to be elevated in osteoarthritis in humans (MacDonald et al. 1994).

In the present study we have also measured osteocalcine concentrations in horse sera. Plasma concentrations of osteocalcine will be evaluated by the optimized standard method

of immunometric assay using a commercially available kit. This standard immunometric assay is considered to have a wide species range.

Bone synthesis is reflected by the release of osteocalcin into serum. An increased concentration of circulating osteocalcin was shown in some humans and dogs (Campion et al. 1989; Nap 1994) even though bone destruction is the key feature of erosive RA, bone repair will also occur, if it is not suppressed by inflammation (Ekenstam et al. 1986; Campion et al. 1989; Poole et al. 1994). However, in this study, the general indication of a lack of increase of osteocalcin suggests that either most of the horses were at an early stage in osteoarthritis or that bone synthesis is impaired in equine OA, possibly as a result of the effects of circulating cytokines; it is well established that both IL-1 β and TNF α inhibit bone formation (Poole and Dieppe 1994). Black et al. (1999) has shown that diurnal rhythms exist for serum OC in adult horses. Therefore, sample timing is an important consideration in equine studies.

Serum osteocalcin concentrations could be related to the age of the horses in the OA groups. This may reflect the severity of the disease, or perhaps more probably the degree of bone change due to a long period of joint disease. It is not clear whether these correlations provide much insight into the consequences of the disease process or eventual bone loss in OA (Graffenau et al. 2000). The lack of a correlation of osteocalcin with erosive changes indicates that osteocalcin levels alter either before or independently of bone changes (Lepage et al. 1991, 2001).

The bone changes may well be detectable after changes to the cartilage have occurred, particularly in erosive disease. It is hoped that further studies on cartilage breakdown products and bone matrix non-collagenous macromolecules in the equine arthritides will give further insight into the pathogenesis of these diseases and may provide useful information for OA in other species.

The measurement of collagen crosslinks, deoxypyridinoline is comparatively more specific to monitor bone resorption of OA. In conclusion, this study demonstrated that determination of collagen cross links products is useful diagnostically. We suggest that they are of sufficient interest to warrant further research into their biological significance and potential for assessing disease severity and progression.

Význam deoxypyridinolinu a osteokalcinu v diagnostice osteoarthritis u koní

V moči koní s osteoartritidou (OA) jsme měřili koncentraci deoxypyridinolinu (Dpd) a osteokalcinu (OC). Tyto látky jsou považovány za diagnostický a prognostický marker kloubních onemocnění u lidí a psů. Testy v séru a moči poskytují neinvazivní alternativu kostní biopsie při studiu metabolismu kostí u koní.

V žádné skupině nebyly v séru zjištěny významné rozdíly v hodnotách osteokalcinu, ale významné rozdíly se objevily v poměru deoxypyridinolinu a kreatininu v normální a OA moči. Ty lze využít při monitoringu terapie a prognózy osteoartrity u koní. Měření spojovacího článku kolagenu, deoxypyridinolinu, je srovnatelně specifitější s ohledem na monitoring resorpce kostí. V práci jsou shrnuty způsoby užití rozdílných markerů kostí ve veterinární medicíně a možnosti v diagnostice a prevenci onemocnění kostí u koní.

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