OsteoArthritis and Cartilage (2006) **14,** 1174–1180

doi:10.1016/j.joca.2006.04.017

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Urine cartilage oligomeric matrix protein (COMP) measurement is useful in discriminating the osteoarthritic Thoroughbreds

K. Misumi Ph.D.†*, M. Tagami D.V.M.‡, T. Kamimura D.V.M.§, D. Miyakoshi D.V.M.†, I. E. Helal D.V.M.†‖, K. Arai D.V.M.† and M. Fujiki Ph.D.†

† *Department of Veterinary Clinical Science, Kagoshima University, Japan*

‡ *Shadai Horse Clinic, Hokkaido, Japan*

§ *Kamimura Race Horse Clinic, Kumamoto, Japan*

‖ *Department of Veterinary Surgery and Radiology, Suez Canal University, Egypt*

Summary

Objective: To quantify the urinary concentration of cartilage oligomeric matrix protein (COMP), and to evaluate the relationship between urinary COMP concentration and the catabolic activity of synovial fluid (SF) in diseased horses.

Methods: COMP in horse urine was detected by immunoblotting with a monoclonal antibody (mAb; 14G4) raised against equine COMP from articular cartilage. Urine and serum samples were obtained from 83 Thoroughbred horses with aseptic joint diseases (AJD, 79 horses) or septic joint diseases (SJD, four horses) at the time of anesthesia induction, and samples of SF were obtained during surgery. Control samples of urine ($n = 111$) were collected from normal horses free of any orthopedic diseases after they had been racing. COMP concentration was determined in all samples using inhibition enzyme-linked immunosorbent assay (ELISA) with mAb 14G4. SF samples were also used for the quantification of gelatinase activity.

Results: Positive bands of COMP fragments were determined on the immunoblots with mAb 14G4. The urinary COMP concentrations in AJD and SJD horses (1.02 ± 0.75 and 1.55 ± 1.17 μg/100 mg creatinine, respectively) were significantly higher than normal (0.57 ± 0.29 μg/100 mg creatinine). In 55 horses with fractures in the AJD group there was a logarithmic relationship ($r = -0.45$, $P < 0.001$) between the urinary and SF COMP measurements, while the urinary COMP level was positively correlated with matrix metalloproteinase (MMP)-2 and -9 activities ($r = 0.30$, $P < 0.05$ and $r = 0.51$, $P < 0.001$, respectively) in SF.

Conclusions: The urinary COMP assay with mAb 14G4 is useful for discriminating horses with osteoarthritis. The higher COMP levels in urine from such horses would be indicative of enhanced proteolytic activity, in addition to the increased COMP levels in the diseased joints.

Key words: COMP, Horse, Osteoarthritis, Urine.

Introduction

Cartilage oligomeric matrix protein (COMP) in synovial fluid (SF) and serum has been universally recognized as a sensitive marker of cartilage degradation in clinical studies of osteoarthritis (OA) and rheumatoid arthritis (RA)[1]. The COMP level in SF correlates positively with antigenic keratan sulfate, and also changes depending on differences in cartilage deterioration in idiopathic osteonecrosis as well as hip OA[2]. Also, the serum level of COMP has been reported to be higher in patients with OA than in healthy volunteers[3]. The serum level of COMP is significantly correlated with the Western Ontario and McMaster Universities index pain scale for the lower limbs, and higher in patients with bilateral knee OA than in unilaterally affected patients[4]. Another study has suggested that serum COMP concentration at the baseline could be useful for predicting the course of OA, progressors showing higher average values than non-progressors[5]. As just above, so far, the serum COMP level could be well indicative of the changeable pathology in human OA, whereas no report has demonstrated whether the higher serum COMP represents the increased COMP molecules in diseased SF or not.

In horses with OA, we have demonstrated augmented degradation of COMP in SF[6,7], and suggested that COMP fragmentation in SF could be useful for monitoring equine OA in combination with enzyme-linked immunosorbent assay (ELISA) measurements[7]. Thereafter, we investigated the clinical usefulness of the serum baseline COMP level as a diagnostic marker of OA in racehorses; however, we did not obtain any clear-cut evidence such as a significant correlation of the COMP level in serum to that in SF, as well as to joint pathology or prognosis. There may be certain reasons why serum COMP may be a less sensitive indicator of joint deterioration in equine OA. Exercise-induced up-regulation of COMP turnover might be one factor that would complicate the detection of changes in serum COMP concentration due to OA pathology in horses. Neidhart *et al.*[8] pointed out that the baseline level of COMP in human marathon runners (before exercise) was significantly higher than in non-running healthy volunteers (controls who had never run in any competitive race, and who were age- and sex-matched).

*Address correspondence and reprint requests to: Kazuhiro Misumi, Ph.D., Department of Veterinary Clinical Science, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan. Tel/Fax: 81-99-285-8730; E-mail: kmisumi@agri.kagoshima-u.ac.jp

Received 9 February 2006; revision accepted 22 April 2006.

We have also obtained evidence that the baseline level of COMP in the serum of Thoroughbreds can increase significantly as a result of more intensive workouts (unpublished data). Our finding that the serum COMP level was increased 1 h after strenuous exercise on a racecourse and then recovered to the baseline within 24 h is also in agreement with previous studies[8,9]. The rapid clearance of COMP from serum might also make it difficult to determine any increase in the serum level specific to joint diseases.

Attenuation of COMP outflow from joint fluid into the blood circulation might be another factor that would obscure any change in the serum COMP concentration due to OA pathology. In horses with OA, increased catabolic activity reflected in the levels of matrix metalloproteinases (MMPs) and inflammatory cytokines has been demonstrated in SF[10], but so far not in serum. Thus, the attenuation of COMP outflow from affected joints might not be due to proteolytic degradation in the blood compartment. We have another hypothesis that the increased amount of COMP fragments released from affected joints could be attenuated principally through dilution with a large volume of blood, and so would not be detectable unless a blood concentrate was examined. If it was possible to quantify COMP using samples of urine, which is a concentrated filtrate of blood, then measurements might be correlated with changes in COMP turnover in response to joint pathology. The urinary level of C-terminal cross-linking telopeptide of type II collagen (U-CTX-II) determined using a monoclonal antibody (mAb) raised against a less-proteolytic linear amino acid peptide has been demonstrated to be the most reliable of several biomarkers of OA[11]. Conveniently, mAb 14G4, which we previously raised against equine cartilage COMP, can recognize the COMP fragments in horse urine[7]. Hypothesizing that urinary COMP would be a more reliable marker of equine OA than serum COMP, we devised a system for assay of urinary COMP using mAb 14G4. The aims of this study were to evaluate the usefulness of urinary COMP measurement for discriminating horses with OA from normal horses, and the relationship between the level of urinary COMP and joint pathology in OA assessed by sampling of SF.

Subjects, materials and methods

SAMPLE COLLECTION

Individual urine and serum samples were obtained from 83 Thoroughbred horses with aseptic or septic joint diseases (AJD and SJD, 79 and four horses, respectively) at the time of anesthesia induction, and samples of SF were obtained at the time of arthroscopic surgery. All the horses were determined to be free of any medical diseases including renal failure and uropathy, based on the preoperative assessment of blood and urine. The surgical cases of AJD comprised 55 cases of intra-articular fracture including secondary degenerative joint disease (DJD) and 24 cases of osteochondrosis (OC; including osteochondritis dissecans (OCD) and bone cyst). Control samples of urine ($n = 111$) were collected from normal horses judged to be free of any orthopedic diseases on the basis of clinical, radiological and ultrasound examinations, after the horses had won a race. The laboratory tests for the urine samples also showed no abnormalities suggesting any renal disorder. The samples were centrifuged to remove debris or cells, and the supernatants were stored at $-70\,^{\circ}C$ until assay. Prior to assay, all SF samples were pretreated with hyaluronidase according to Neidhart *et al.*[12] (characteristics of the horses are summarized in Table I).

Table I
Characteristics of the horses, and diagnosis of the affected joints

Normal horses ($n = 111$)	
Age (years, mean ± 1 SD)	2.5 ± 1.1
Gender	
Male	71
Female	40
Joint diseased horses ($n = 83$)	
Age (years, mean ± 1SD)	2.2 ± 1.2
Gender	
Male	52
Female	37
AJD ($n = 79$)	
Fracture	
Carpus	37
Hind fetlock	14
Fore fetlock	3
Tarsus	1
OC	
Tarsus	19
Stifle	5
SJD ($n = 4$)	
Shoulder	1
Fore fetlock	1
Hind fetlock	1
Tarsus	1

The control samples were obtained from the normal horses without any joint diseases. Diseased samples were divided into two categories such as AJD and SJD.

ELECTROPHORESIS AND IMMUNOBLOTTING OF URINE SAMPLES

Following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis on 15% polyacrylamide gel containing 8 M urea as reported previously[13], the gels were electrotransferred onto polyvinyl difluoride membranes. After blocking, mAb 14G4, diluted 1:10,000 in 5% skimmed milk in Tris-buffered saline containing 0.05% Tween 20 (TBS/Tween), was applied to the membrane. Positive binding to COMP was detected using alkaline phosphatase (AP)-conjugated goat anti-mouse IgG antibody (A36882) diluted 1:10,000 in 2% skimmed milk in TBS/Tween, and demonstrated by development of the reaction with a substrate (BCIP/NBT, Sigma Fast/B-56552).

INHIBITION ELISA

COMP concentration in urine, SF, and serum was analyzed by inhibition ELISA with mAb 14G4 directed against equine COMP designed according to previous protocols[6,7] with some modifications. In brief, 50 μl of purified horse COMP antigen in a coating buffer (20 mM sodium carbonate, 20 mM sodium bicarbonate, 0.002% sodium azide, pH 10) was placed in each well at 5 μg/ml, and then incubated for 2 h at room temperature followed by overnight at 4 °C. Seventy microliters of diluted standards (range: 13.5–0.01 μg/ml) and the samples (final dilutions: urine 1/5, SF 1/70, serum 1/20) were mixed with the same volume of mAb 14G4 (final dilution: 1/100,000) in phosphate-buffered saline containing 0.05% Tween 20 (PBS/Tween),