OsteoArthritis and Cartilage (2006) **14,** 299–301

doi:10.1016/j.joca.2005.10.005

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Brief report
Raised chondroitin sulfate epitopes and hyaluronan in serum from rheumatoid arthritis and osteoarthritis patients

P. Pothacharoen†, S. Teekachunhatean‡, W. Louthrenoo§, W. Yingsung†, S. Ong-Chai†, T. Hardingham‖ and P. Kongtawelert†*

† *Thailand Excellence Centre for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

‡ *Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

§ *Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

‖ *UK Centre for Tissue Engineering, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, UK*

Summary

Objectives: Serum hyaluronan (HA) and chondroitin sulfate (CS) epitopes WF6 and 3B3 (+) were determined to investigate disease association in patients with osteoarthritis (OA), rheumatoid arthritis (RA) and healthy controls.

Methods: Specific assays for HA and CS epitopes WF6 and 3B3 (+) were established and applied to a cross-sectional study of serum samples from patients (96 OA, 57 RA and 50 healthy controls).

Results: Both CS epitopes were increased in serum of many OA and RA patients and average levels were significantly above in healthy controls. In contrast serum HA was increased in RA, but only in few OA patients.

Conclusions: CS epitopes WF6 and 3B3 (+) are raised in serum of patients with both OA and RA and were thus distinct from serum HA. The results suggest that OA may be detected systemically as well as RA. The range of levels of CS epitopes detected in OA and RA was wide and correlation with any aspect of disease activity is yet to be determined.

Key words: Biomarker, Chondroitin sulfate epitope, Degenerative joint diseases.

Introduction

The diagnosis of osteoarthritis (OA) is generally based upon clinical and radiographic changes that occur in the later stages of the disease. There is considerable interest in identifying better criteria for diagnosis and methods for monitoring disease activity and progression as such methods would not only enable earlier diagnosis, but would be important tools in the development of better treatments[1]. Measurement of macromolecules released from cartilage into synovial fluid of OA joints has been investigated to follow the process of cartilage damage and destruction[2]. However, there is limited access to synovial fluid and if characteristic changes were identified in serum, they would have much wider application. In the present study we have established assays for the investigation of chondroitin sulfate (CS) epitopes in serum samples of patients with joint disease. A novel monoclonal antibody (WF6), which recognises a native epitope in CS chains was evaluated together with a monoclonal antibody 3B3, which recognises unsaturated terminal chondroitin 6-sulfate after chondroitinase ABC digestion[1,3].

*Address correspondence and reprint requests to: P. Kongtawelert, Thailand Excellence Centre for Tissue Engineering, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. Tel/Fax: 66-53-894188; E-mail: pkongtaw@mail.med.cmu.ac.th

Received 22 June 2004; revision accepted 8 October 2005.

Materials and methods

Assay methods were optimised to investigate these CS epitopes in normal, OA and RA serum samples. The results were also compared with measures of serum hyaluronan (HA), which has been shown to be elevated in serum in patients with inflammatory joint disease such as RA[4–7]. These assays were applied to the cross-sectional analysis of serum from rheumatoid arthritis (RA) and OA patients and healthy controls in order to evaluate their potential as biomarkers for joint diseases. OA patients were recruited who were of either sex, of age over 40 and were suffering from unilateral (16%) or bilateral (84%) knee OA and satisfied the American College of Rheumatology (ACR) classification criteria for OA of the knee[8] of more than 3 months duration (3.94 ± 2.86 years). The Kellgren and Lawrence X-ray grades were 2% grade 1, 7% grade 2, 16% grade 3 and 75% grade 4. Patients took paracetamol 18.9 ± 14.7 tablets (300 mg)/ week. The median (range) of 100 mm/visual analogue scale (VAS), Western Ontario and McMaster University Osteoarthritis Index VAS (WOMAC) score and Lequesne's index are 66 (20–100), 46 (18–96) and 14 (7–21), respectively. Patients with RA of the knee were diagnosed on the basis of clinical

symptoms, examination and radiographic findings and fulfilled the ACR criteria of RA[9]. RA patients' ages were 49.6 ± 12.5 years (range 24–75), and duration of diseases was 7.9 ± 6.6 years. The disease classifications[10] were 24 in class I, 64 in class II and six in class III, and none were in class IV. Sixty-six patients (70%) were rheumatoid factor (RF) positive. Patients were taking chloroquine, methotrexate and sulfasalazine in 71, 52 and two cases, respectively. No patient took prednisolone. For rheumatological evaluation blood collection and radiographs were completed on the morning of the same day (between 08:00 and 10:00 a.m. or 3 and 5 h after waking up in the morning). For normal serum fasting-morning serum samples were collected from 50 healthy blood donors, aged 41–75 years. All subjects gave their informed consent before participation and the study received ethical approval from Chiang Mai University. They were checked to be free from diseases of the joints, bones, liver, endocrine system, or other chronic disorders and none were currently taking any medication known to modify arthritic diseases or influence joint metabolism. Sera were stored at −20 °C before analysis. A competitive immunoassay with the monoclonal antibody 3B3 was developed to analyse human serum for chondroitin 6-sulfate 3B3 (+) epitope after digestion with chondroitinase ABC. Human serum samples were also assayed by a competitive immunoassay with monoclonal antibody WF6, which recognises native epitopes in CS chains without chondroitinase ABC digestion. Serum HA was also determined using an enzyme-linked immunosorbent assay (ELISA) based assay using biotinylated HA binding proteins.

Results and discussion

The analysis of HA in serum of patients with joint diseases has shown increased levels particularly in those with RA[4–6]. It has also been reported that in patients with knee OA a higher value at onset was predictive of a more rapid progression[7]. In this study the results with a newly developed assay were comparable with previous results amongst clinically defined patient groups showing many RA patients (23/57) and also a few OA patients (17/96) with high serum HA.

It is interesting to compare the results for serum HA with the results for the CS epitopes, 3B3 (+) and WF6. The 3B3 (+) epitope may provide a measure of the mobilisation of tissue proteoglycans containing chondroitin 6-sulfate. This might be expected to parallel the release of HA. The results (Fig. 1) showed that indeed many RA samples contained very high levels of 3B3 (+) epitope, however, there was no significant correlation between serum HA and serum 3B3 (+) and they varied independently of each other. The OA sera also contained levels of 3B3 (+) epitope significantly higher than normal. Clearly, tissue mobilisation of HA, which might be caused by inflammation, is driven by different processes from those that raise serum 3B3 (+). The native CS epitope detected by monoclonal antibody WF6 was also raised above normal in RA and OA serum. This might imply that 3B3 (+) and WF6 detect the same components in serum, however, analysis of the results showed that they also varied independently of each other. They are thus detecting unlinked features of the CS structures in serum. As there are many different CS proteoglycans in the body, the epitopes may be part of different proteoglycans from different tissue sources. The high levels of WF6 epitope in RA and OA may reflect an increase in synthesis in joints or other tissues, or its preferential release into serum. The investigation of another CS epitope (846)

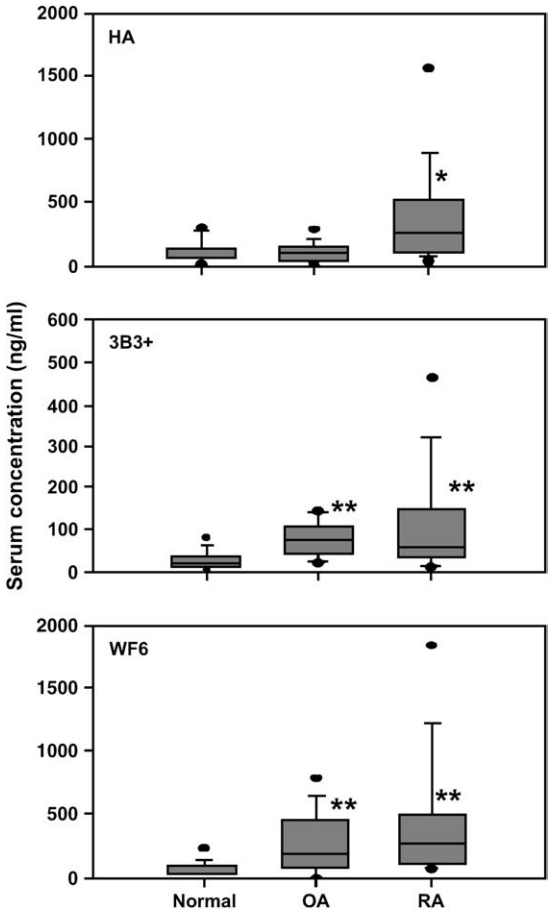

Fig. 1. Serum concentration of HA and CS epitopes WF6 and 3B3 (+) in OA, RA and healthy controls (normal). Boxes represent median and the interquartile range, between 5th and 95th quartile with error bars. Statistically significant difference ($P < 0.001$ shown with double asterisks) relative to the median of the normal serum.

has been suggested to reflect an increase in synthesis and has been reported to be highest in serum of early RA patients with slow disease progression[11]. An increase in WF6 epitope may also reflect an anabolic response and it may be helpful in diagnosis or prognosis.

The 3B3 epitope is a 6-sulfated terminal disaccharide on CS[1]. The antibody reacts with both native terminal disaccharides (denoted 3B3 (−)) and after chondroitinase digestion with reduced terminal disaccharides (denoted 3B3 (+))[1,3]. However, the 3B3 (−) epitope is uncommon in native CS chains[12] and the detectible epitope was increased after digestion by 20–100 fold in serum samples. The 3B3 (+) assay developed in this study with monoclonal antibody (mAb) thus recognises unsaturated terminal 6-sulfated disaccharide structures remaining attached to protein after chondroitinase ABC digestion. As 3B3 is an immunoglobulin M (IgM), it is likely to be more reactive to polyvalent antigens and proteoglycan fragments with several chains and several epitopes may be more competitive in the assay[13]. The assay of 3B3 (−) in serum has been reported to show levels lower than normal in early RA[14]. The serum results with 3B3 (+) and WF6 may, therefore, reflect systemic changes that accompany joint disease, or they may be selectively contributed to by joint components if these are particularly reactive in the assays and enter the circulation without efficient removal.

As aggrecan degradation products from cartilage are likely to be present in serum and to be polyvalent, the detection with 3B3 (+) may selectively provide a measure of catabolism in cartilaginous tissues.

The circulating level of CS epitopes is a balance of several factors; the release from tissues, the uptake within the lymphatics and their removal from serum by the liver once they have entered the circulation. Serum detection of WF6 and 3B3 (+) may, therefore, be influenced by other factors that compromise liver uptake or kidney excretion of CS proteoglycan metabolites and care was taken in this study to exclude any patients with other non-joint related pathology from the study.

The determination of the CS epitopes 3B3 (+) and WF6 in serum show increases in groups of both OA and RA patients and they may therefore prove useful in the differential diagnosis and monitoring of joint diseases. Whilst mean serum concentrations of 3B3 (+) and WF6 epitopes were raised in these disease groups, the distribution of levels found was broad and it is interesting that some patients were in the normal range. There were no obvious clinical criteria that distinguished those with high values from those with low values in this cross-sectional study and whether it relates to disease activity or progression will need to be evaluated in a more detailed longitudinal study.

Acknowledgements

The Thailand Research Fund (Basic Research Grant to PK), The Royal Golden Jubilee Ph.D. Program, Grant No. PHD/0121/2544 (to PP) and the National Research Council of Thailand (Research Programme of Drug, Chemical, Medical Material and Equipment) jointly funded this work.

References

1. Caterson B, Mahmoodian F, Sorrell JM, Hardingham T, Bayliss MT, Carney SL, *et al*. Modulation of native chondroitin sulphate structure in tissue development and in disease. J Cell Sci 1990;97:411–7.
2. Lohmander S. Markers of cartilage metabolism in arthrosis. Acta Orthop Scand 1991;62:623–32.
3. Caterson B, Hughes CE, Johnstone B, Mort JS. Immunological markers of cartilage proteoglycan metabolism in animal and human osteoarthritis. In: Kuettner K, Schleyerback R, Peyron J, Hascall V, Eds. Articular Cartilage and Osteoarthritis. New York: Raven Press 1992:415–426.
4. Paimela L, Heiskanen A, Kurki P, Helve T, Leirisalo-Repo M. Serum hyaluronate levels as a predictor of radiologic progression in early rheumatoid arthritis. Arthritis Rheum 1991;34:815–21.
5. Laurent TC, Fraser JRE, Laurent UBG, Engstrom-Laurent AE. Hyaluronan in inflammatory joint disease. Acta Orthop Scand 1995;66:116–20.
6. Fex E, Eberhardt K, Saxne T. Tissue derived macromolecules and markers of inflammation in serum in early rheumatoid arthritis: relationship to development of joint destruction in hands and feet. Br J Rheumatol 1997;36:1161–5.
7. Sharif M, George E, Shepstone L, Knudson W, Thonar EJ, Cushnaghan J, *et al*. Serum hyaluronic acid as a predictor of disease progression in osteoarthritis of the knee. Arthritis Rheum 1995;38:760–7.
8. Altman R, Asch E, Bloch D, *et al*. Development of criteria for the classification and reporting of osteoarthritis. Arthritis Rheum 1986;29:1039–49.
9. Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
10. Hochberg MC, Chan RW, Dwosh I, *et al*. The American College of Rheumatology 1991 revised criteria for the classification of global functional status in rheumatoid arthritis. Arthritis Rheum 1992;35:498–502.
11. Hardingham TE. Immunochemical methods in cartilage research. In: Maroudas A, Keuttner K, Eds. Methods in Cartilage Research. London: Academic Press 1991: 155–164.
12. Mansson B, Carey D, Alini M, Ionescu M, Rosenberg LC, Poole AR, *et al*. Cartilage and bone metabolism in rheumatoid arthritis. Differences between rapid and slow progression of disease identified by serum markers of cartilage metabolism. J Clin Invest 1995;95:1071–7.
13. Plaas AH, West LA, Wong-Palms S, Nelson FR. Glycosaminoglycan sulfation in human osteoarthritis. Disease related alterations in the non-reducing termini of chondroitin and dermatan sulphate. J Biol Chem 1998; 273:12642–9.
14. Middleton J, White S, Parry E, Jackson J, Dixey J, Ashton B. Changes in serum chondroitin sulphate epitopes 3B3 and 7D4 in early rheumatoid arthritis. Rheumatology 1999;38:837–40.