Research article

Serum cathepsin K levels of patients with longstanding rheumatoid arthritis: correlation with radiological destruction

Martin Skoumal1,2, Günther Haberhauer1, Gernot Kolarz1, Gerhard Hawa3, Wolfgang Woloszczuk4 and Anton Klingler5

1Institut für Rheumatologie der Kurstadt Baden in Kooperation mit der Donauuniversität Krems, Austria
2Rheumasonderkrankenanstalt der SVA der gewerblichen Wirtschaft, Baden, Austria
3Biomedica Medizinprodukte GmbH & CO KG, Vienna, Austria
4L Boltzmann Institut für experimentelle Endokrinologie, Vienna, Austria
5Theoretical Surgery Unit, Department of General and Transplant Surgery, University Hospital, Innsbruck, Austria

Corresponding author: Martin Skoumal, martin.skoumal@a1.net

Received: 16 Aug 2004 Revisions requested: 22 Sep 2004 Revisions received: 3 Oct 2004 Accepted: 11 Oct 2004 Published: 10 Nov 2004

Arthritis Res Ther 2005, 7:R65-R70 (DOI 10.1186/ar1461)
© 2004 Skoumal et al., licensee BioMed Central Ltd.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is cited.

Abstract

Cathepsin K is a cysteine protease that plays an essential role in osteoclast function and in the degradation of protein components of the bone matrix by cleaving proteins such as collagen type I, collagen type II and osteonectin. Cathepsin K therefore plays a role in bone remodelling and resorption in diseases such as osteoporosis, osteolytic bone metastasis and rheumatoid arthritis. We examined cathepsin K in the serum of 100 patients with active longstanding rheumatoid arthritis. We found increased levels of cathepsin K compared with a healthy control group and found a significant correlation with radiological destruction, measured by the Larsen score. Inhibition of cathepsin K may therefore be a new target for preventing bone erosion and joint destruction in rheumatoid arthritis. However, further studies have to be performed to prove that cathepsin K is a valuable parameter for bone metabolism in patients with early rheumatoid arthritis.

Keywords: bone remodelling, cathepsin K, osteoclast activation, rheumatoid arthritis

Introduction

Progressive bone and cartilage destruction in arthritic joints leads to irreversible joint destruction, and subsequently to functional declines and work disability [1,2]. New biomarkers such as cartilage oligomeric matrix protein [3,4], osteoprotegerin [5-7] or receptor activator of NF-κB ligand [8-10] have been developed to describe the local bone and cartilage process in affected joints.

Cathepsin K is a cysteine protease that plays an essential role in osteoclast function and in the degradation of protein components of the bone matrix. It is produced by bone resorbing macrophages and synovial fibroblasts, and it cleaves proteins such as collagen type I, collagen type II and osteonectin [11]. Cathepsin K therefore plays a role in bone remodelling and resorption in diseases such as osteoporosis, osteolytic bone metastasis and rheumatoid arthritis (RA) [12,13].

Cathepsin K is a tissue-specific protease associated with pycnodysostosis, a rare genetic disorder that manifests itself in bone abnormalities such as short stature, acroosteolysis of distal phalanges and skull deformities [14,15]. Cathepsin K knockout mice develop an osteopetrosis. Inhibition of cathepsin K may therefore prevent bone resorption, as could be demonstrated in bone metastasis from breast cancer [16]. Osteoprotegerin has been shown to inhibit the expression of cathepsin K, the main enzyme involved in bone resorption.

The aim of this study was to measure serum levels of cathepsin K in RA and to prove that cathepsin K is a parameter of bone remodelling and resorption in a nonselected cohort of patients with longstanding RA. This patient group shows a variation of age, inflammatory level and Larsen score. We divided this cohort into different groups, according to age, inflammatory level, disease-modifying antirheumatic drug

CRP = C-reactive protein; DMARD = disease-modifying antirheumatic drug; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; NF = nuclear factor; RA = rheumatoid arthritis.
(DMARD) therapy, radiological progression and disease activity, to verify cathepsin K as an age-independent and laboratory inflammatory parameter-independent protease.

**Materials and methods**

Serum levels of cathepsin K were measured in the sera of 100 patients suffering from RA according to the criteria of the American Rheumatism Association [17]. Clinical and laboratory data are presented in Tables 1 and 2. The control group consisted of nonselected healthy blood donors from a central blood bank (n = 50; 21 female, 29 male) aged 18–65 years.

Most of the patients received DMARDs. The most frequently used DMARD was methotrexate, followed by leflunomide, sulfasalazopyrine and gold. Furthermore, azathioprine and chloroquine but no biological therapy were prescribed (Table 3).

Each examination consisted of a full interview, the assessment of functional disability and a standardised physical examination, which included a joint examination for tenderness (Ritchie score), pain on motion, soft tissue swelling, 44-swollen joint count and swollen proximal interphalangeal score [18,19].

The disease activity of RA was measured by the disease activity score (≤ 2.4, low activity; > 2.5 and ≤ 3.7, mean activity; >3.7, high activity). The radiological progression in RA was calculated by the Larsen score [20].

The blood examination at each visit consisted of the determination of cathepsin K, the erythrocyte sedimentation rate, the haemoglobin level, the thrombocyte count, the serum rheumatoid factor (RapiTex® RF; Dade Behring, Liederbach, Germany), antinuclear antibodies (indirect immunofluorescent technique, ANA Fluor Kit 240®; Diasorin, Stillwater, MN, USA) and C-reactive protein (CRP) (Rheumajet CRP®; Biokit, Barcelona, Spain).
The variables of age, sex, duration of disease, visual analogue scale of general health and morning stiffness, treatment with DMARDs and reason for their discontinuation, and the Steinbrocker stage [21] were also recorded. Serum was obtained in the morning from the routinely taken blood samples and was centrifuged immediately. The samples were kept at -80°C prior to determination of cathepsin K. The serum used for the measurement of cathepsin K was the remainder from routinely drawn blood examinations on the day of hospitalisation; no examination was performed only for quantification of cathepsin K. Clinical data were used from a database developed for the long-term observation of patients with RA in our clinic.

An enzyme immunoassay for cathepsin K developed by Biomedica Austria (Vienna, Austria) was used. The Cathepsin K test kit is an enzyme immunoassay designed to determine cathepsin K directly in biological fluids (serum, plasma, cell culture supernatants). The ELISA used in this study is based on antibodies specific for amino acids 1–20 and amino acids 196–210 of the mature enzyme. The antibodies were produced by immunisation of sheep with peptides of that amino acid sequence coupled to Keyhole Limpet Hämocyanine (primary immunisation, 0.5 mg; boost, 0.25 mg). Antisera were purified using the biotinylated peptides coupled to streptavidin sepharose (Amersham-Pharma Biotech Ltd, Little Chalfont, UK). A synthetic cathepsin K (Pichem GmbH, Graz, Austria) was used as the calibrator. Signal generation was accomplished by labelling with horseradish peroxidase.

Briefly, the assay procedure consisted of incubating 50 µl sample with 200 µl horseradish peroxidase-labelled detection antibody on capture antibody precoated plates overnight at room temperature. After a washing step to remove unbound detection antibody, tetramethyl benzidine was added as the substrate. The reaction was stopped after 30 min by adding 50 µl of 0.9% H₂SO₄. The yellow colour that is directly proportional to the amount of cathepsin K present in the sample was measured on a standard microplate reader at 450 nm with 620 nm as the reference. The detection limit of the assay was calculated as 1.1 pmol/l (0 standard + 5 × standard deviation).

No cross-reactivity to cathepsin E, cathepsin D, cathepsin B and cathepsin L or rheumatoid factors was detected.

Statistical methods included Spearman correlation analysis, the Wilcoxon two-sample test the Kruskal–Wallis test and analysis of variance, if appropriate. P < 5% was considered statistically significant.

Results

The cathepsin K serum levels of the patients with RA (median first–third quartile range, 54.8 pmol/l) compared with the healthy control group (median first–third quartile range, 12.7 pmol/l) were significantly elevated (P = 0.0003) (Table 4).

The Larsen score ranged from 0 to 164 (median score, 39). The Spearman rank correlation showed a statistically significant correlation between cathepsin K and the Larsen score (P = 0.004). The highest levels of cathepsin K were observed in patients with the highest Larsen scores. We divided the cohort into three Larsen groups with equal numbers of patients (Larsen score < 18 points, Larsen score between 19 and 74 points, and Larsen score ≥ 75 points). Cathepsin K levels showed an increase with the augmentation of radiological destruction (P = 0.035) (Table 5).

Cathepsin K seems to be independent or only weakly correlated with laboratory inflammation parameters. It was not associated with CRP (P = 0.27), but weak correlations were found with the erythrocyte sedimentation rate (P = 0.03) and the disease activity score of the whole cohort (P = 0.04). However, the division of the disease activity score into three groups with low activity, medium activity and high activity did not show any difference. We could not find any correlation with sex and age (whole group/division into two patient groups ≤ 65 years and ≥ 66 years, P = 0.32), whereby the two groups were comparable in disease activity (3.53 versus 3.12), laboratory parameters (CRP, 25.4 mg/l versus 25.9 mg/l), clinical score (Ritchie score, 14 versus 9) and radiological score (Larsen score, 47 versus 62).

The most frequently used DMARD was methotrexate (n = 42), followed by leflunomide (n = 10) and sulfasalazine (n = 10). Twenty-two patients had no DMARD at the time of examination (Table 3). The lowest cathepsin K levels were evident in the leflunomide group, but no significant difference between these groups could be demonstrated.

Discussion

Bone resorption and formation is a well-balanced system and is mediated by osteoclasts. Cathepsin K is essential for bone resorption, which depends on the production of cathepsin K by osteoclasts and its secretion into the extracellular department. This leads to a degradation of the organic matrix between the osteoclasts and the bone surface [22]. In vivo the activation of cathepsin K occurs intracellularly, before secretion into the resorbing lacunae and the onset of bone resorption, whereby local factors may regulate the processing of procathespin K to mature cathepsin K [23]. In accordance with this, synovial fibroblasts are also involved in joint destruction and in the pathogenesis of RA. Hou and colleagues found that cathepsin K has a potent aggrecan-degrading activity, whereby the aggrecan cleavage products increase the collag enolytic effects of this protease on collagen type I and type II. They were able
to show that cathepsin K is also a critical protease in cartilage degradation by synovial fibroblasts [24]. Increased expression of cathepsin K around lymphocytic infiltrates in synovial tissue seems to facilitate the movement of mononuclear cells through the perivascular matrix [25]. Proinflammatory cytokines such as IL-1β and tumour necrosis factor alpha influence the expression of cathepsin K. Its overexpression in the rheumatoid synovium, induced by IL-1β and tumour necrosis factor alpha due to the increase of cathepsin K-expressing cells, proves this protease to be a valuable tool for bone research, and cathepsin K also may become a new and highly specific biomarker for RA [26].

Wittrant and colleagues demonstrated osteoprotegerin to be an inhibitor of cathepsin K. Osteoprotegerin is an osteoblast-secreted decoy receptor that inhibits osteoclast differentiation and activation. Human osteoprotegerin inhibits cathepsin K and tartrate-resistant acid phosphatase, both osteoclast markers, but stimulates the expression of tissue inhibitor of metalloproteinases-1 [29]. These results are a further step in the development of new therapies for the prevention of bone destruction.

In the synovium of RA, the cathepsin K protein was localised in synovial fibroblasts, stromal multinucleated giant cells and CD68+ macrophage-like synoviocytes. Highly
interesting is the expression of cathepsin K by fibroblasts and giant cells at sites of cartilage erosions. This was two to five times higher compared with osteoarthritic synovium. In normal synovium, cathepsin K expression was not increased and was restricted to fibroblast-like cells [26, 30-32]. The overexpression of cathepsin K in RA synovia proves that this protease is responsible for the degradation of articular tissue in rheumatoid joints and in normal synovial tissue.

To our knowledge, no study has previously investigated the serum levels of cathepsin K in RA. Our results demonstrate that cathepsin K is elevated in the serum of patients with RA compared with that of a healthy control group (Table 4). The upregulation of cathepsin K and the correlation with the Larsen score as a parameter for radiological changes (Table 5) mirrors the destruction of bone structures in inflammatory diseases like RA. The measurement of cathepsin K seems an inexpensive tool that is independent of CRP and shows only a weak correlation with the erythrocyte sedimentation rate.

Further studies should investigate whether elevated cathepsin K levels precede osteosial destruction or whether they occur as result of them. In the first case, determination of cathepsin K could be an important additional tool to decide on aggressive forms of disease-modifying antirheumatic therapies.

Conclusion
This is the first study that demonstrates increased cathepsin K levels in the serum of patients with RA. As could be shown in the synovia of RA, the elevated serum levels of this protease are significantly correlated with the joint destruction, which in this study was assessed by the Larsen score. Cathepsin K seems to be a valuable parameter for the assessment of bone metabolism in patients with established RA and its measurement will probably contribute to developing targeted therapies for the prevention of further bone destruction. However, more studies need to be performed to verify the presence of cathepsin K in patients with early RA and its value as a prognostic factor for bone destruction in RA.

Competing interests
Dr G Hawa and Prof. W Woloszczuk are members of BIO-MEDICA who developed the Cathepsin K kit, but they did not receive any financial benefits.

Authors’ contributions
MS is the corresponding author, and GH and GK are coauthors of the manuscript. GH and WW developed the cathepsin K ELISA kit. AK performed the statistical analysis.

References
1. Pincus T, Callahan LF, Sale WG, Brooks AL, Payne LE, Vaughn WK: Severe functional declines, work disability, and increased mortality in seventy-five rheumatoid arthritis patients studied over nine years. Arthritis Rheum 1984, 27:864-872.
2. Mulsini D, Fitzgerald O, Bresnihan B: Clinical improvement and radiological deterioration in rheumatoid arthritis: evidence that the pathogenesis of synovial inflammation and articular erosion may differ. Br J Rheumatol 1996, 35:1263-1268.
3. Skoumal M, Kolarz G, Klinger A: Serum levels of cartilage oligomeric matrix protein (COMP): a predicting factor and a valuable parameter for disease management in rheumatoid arthritis. Scand J Rheumatol 2003, 32:156-181.
4. Skoumal M, Haberhauer G, Hertag J, Kittl EM, Bauer K, Dunky A: Serum levels of cartilage oligomeric matrix protein (COMP) are elevated in rheumatoid arthritis, but not in inflammatory rheumatic diseases as psoriatic arthritis, reactive arthritis, Raynaud’s syndrome, scleroderma, systemic lupus erythematosus, vasculitis and Sjögren’s syndrome. Arthritis Res Ther 2004, 6:73-74.
5. Schett G, Redlich K, Smolen JS: The role of osteoprotegerin in arthritis. Arthritis Res Ther 2003, 5:239-245.
6. Lacey DL, Timms E, Tan HL, Kelly MJ, Dunston CR, Burgess T, Elliott R, Colombo A, Elliott G, Scully S, et al: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998, 93:165-176.
7. Skoumal M, Kolarz G, Woloszczuk W, Hawa G, Klinger A: Serum osteoprotegerin but not receptor activator of NF-κB B Lig and correlates with the Larsen score in rheumatoid arthritis. Ann Rheum Dis 2004, 63:216-217.
8. Hofbauer LC, Heufelder AC: Role of receptor activator of nuclear factor-κB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001, 79:243-253.
9. Takayanagi H, Iizuka H, Fujii T, Nakagawa T, Yamamoto A, Miyazaki T, Koshihara Y, Oda H, Nakamura K, Tanaka S: Involvement of receptor activator of nuclear factor-κB ligand osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 2000, 43:259-269.
10. Hawa G, Brinskelle-Schmal N, Glatz K, Maintzen S, Woloszczuk W: Immunoassay for soluble RANKL (receptor activator of NF-κB ligand) in serum. Clin Lab 2003, 49:461-463.
11. Hou WS, Li Z, Gordon RE, Chan K, Klein MJ, Levy R, Keyszer M, Keyszer G, Bromme D: Cathepsin K is a critical protease in synovial fibroblast-mediated collagen degradation. Am J Pathol 2001, 159:2167-2177.
12. Goto T, Yamao T, Tanaka K: Cathepsins in the osteoclast. J Electron Microsc (Tokyo) 2003, 52:551-558.
13. Rinman DJ, McClung HA, Dodds RA, Hwang SM, James IE, Drake FH, Gowen M: Biosynthesis and processing of cathepsin K in cultured human osteoclasts. Bone 2001, 28:282-289.
14. Motyckova G, Fisher DE: Pycnodysostosis: role and regulation of cathepsin K in osteoclast function and human disease. Curr Mol Med 2002, 2:407-421.
15. Singh AR, Kaur A, Anand NK, Singh JR: Pycnodysostosis visceral manifestations and simian crease. Indian J Pediatr 2004, 71:453-455.
16. Ishikawa T, Kamiyama M, Tani-Ishii N, Suzuki H, Ichikawa Y, Hamaguchi Y, Momiyama N, Shimada H: Inhibition of osteoclast differentiation and bone resorption by cathepsin K antisense oligonucleotides. Mol Carcinog 2001, 32:84-91.
17. Annett FC, Edworthy SM, Bloch DA, McShane DJ, Sills JF, Wolfe F, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988, 31:315-324.
18. Ritchie DM, Boyle JA, Moynes NM, Jassam MK, Dalakos TG, Grieve son P: Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. Q J Med 1968, 41:393-406.
19. Smolen JS, Breedveld FC, Eberl G: Validity and reliability of the twenty-eight-joint count for the assessment of rheumatoid arthritis. Arthritis Rheum 1998, 40:38-43.
20. Larsen A, Dale K, Eek M: Radiographic evaluation of rheumatoid arthritis and related conditions by standard reference films. Acta Radiol Diagn (stockh) 1977, 18:491-491.

Available online http://arthritis-research.com/content/7/1/R65
21. Steinbrocker O, Traeger CH, Battermann RC: Therapeutic criteria in rheumatoid arthritis. *J Am Med Assoc* 1949, 140:659-663.

22. Troen BR: The role of cathepsin K in normal bone resorption. *Drug News Perspect* 2004, 17:19-28.

23. Dodds RA, James IE, Riemann D, Ahern R, Hwang SM, Connor JR, Thompson SD, Veber DF, Drake FH, Holmes S, et al.: Human osteoclast cathepsin K is processed intracellularly prior to attachment and bone resorption. *J Bone Miner Res* 2001, 16:478-486.

24. Hou WS, Li Z, Buttner FH, Bartnik E, Bromme D: Cleavage site specificity of cathepsin K toward cartilage proteoglycans and protease complex formation. *Biol Chem* 2003, 384:891-897.

25. Hummel KM, Petrow PK, Franz JK, Maller-Ladner U, Aicher WK, Gay RE, Brämme D, Gay S: Cysteine protease cathepsin K mRNA is expressed in synovium of patients with rheumatoid arthritis and is detected at sites of synovial bone destruction. *J Rheumatol* 1998, 25:1887-1894.

26. Hou WS, Li Z, Keyszer G, Weber E, Levy R, Klein MJ, Gravallese EM, Goldring SR, Bromme D: Comparison of cathepsin K and S expression within the rheumatoid and osteoarthritic synovium. *Arthritis Rheum* 2002, 46:663-674.

27. Votta BJ, Levy MA, Badger A, Bradbeer J, Dodds RA, James IE, Thompson S, Bossaard MJ, Carr T, Connor JR, et al.: Peptide aldehyde inhibitor of cathepsin K inhibit bone resorption both in vitro and in vivo. *J Bone Miner Res* 1997, 12:1396-1406.

28. Tavares FX, Boncek V, Deaton DN, Hassell AM, Long ST, Miller Payne AA, Miller LR, Shewchuk LM, Wolfe K, et al.: Design of potent, selective and orally bioavailable inhibitors of cysteine protease cathepsin K. *J Med Chem* 2004, 47:588-599.

29. Wittrant Y, Couillaud S, Theoleyre S, Dunstan C, Heymann D, Redini F: Osteoprotegerin differentially regulates protease expression in osteoclast cultures. *Biochem Biophys Res Commun* 2002, 293:38-44.

30. Kaneko M, Tomita T, Nakase T, Ohsawa Y, Seki H, Takeuchi E, Takano H, Shi K, Takaki K, Kominami E, et al.: Expression of proteinases and inflammatory cytokines in subchondral bone regions in the destructive joint of rheumatoid arthritis. *Rheumatology (Oxford)* 2001, 40:247-255.

31. Li Z, Hou WS, Escalante-Torres CR, Gelb BD, Bromme D: Collagenase activity of cathepsin K depends on complex formation with chondroitin sulfate. *J Biol Chem* 2002, 277:28669-28676.

32. Li Z, Yasuda Y, Li W, Bogoy M, Katz N, Gordon RE, Fields GB, Bromme D: Regulation of collagenase activities of human cathepsins by glycosaminoglycans. *J Biol Chem* 2004, 279:5470-5479.