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Matrix metalloproteinases in patients with osteoarthritis of the hip

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Abstract In 78 patients undergoing a total hip replacement we examined the production of matrix metalloproteinases (MMP) by the fibroblasts from the connective tissue of the acetabulum; we then correlated these findings with clinical and radiological characteristics of the same patients. In 53 patients only MMP-2 was produced; in 15 cases MMP-1, -2 and -3 were present; and in 10 cases not only MMP-1, -2, -3 but also MMP-9. Significant differences among the clinical and radiological parameters were found in the 3 subsets. A positive correlation between the production of MMP-9 and a rapid destruction of the hip joint was found.

Résumé Nous avons examiné la production de métalloprotéinases (MMP) par des fibroblastes dans 78 patients souffrant d'une ostéo-arthrite de la hanche. La production de MMP fut classée en 3 sous-sembles: 53 patients dans lequel seulement des MMP-2 furent détectées; 15 patients dans lequels des MMP-1, -2 et –3 étaient présentes; et 10 patients où des MMP-1, -2, -3 et aussi des MMP-9 furent détectées. Des différences importantes furent découvertes pour des paramètres cliniques et radiologiques parmi les sous-sembles. Il y'a une correlation positive entre la production de MMP-9 et une destruction rapide de la hanche.

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Introduction

The natural history of osteoarthritis (OA) of the hip is variable. It has been suggested that different subsets with distinct etiological pathways [16] may exist. Radiographic classifications of OA of the hip are most commonly used by clinicians [1,2]. However, radiological assessment has limitations in terms of prognostication of disease progression. In this respect, a biochemical classification might be useful for disclosing subtypes with different biological conditions leading to joint destruction.

Matrix metalloproteinases (MMPs), a family of enzymes capable of degrading extracellular matrix at physiological pH, are thought to play an important role in degradation of cartilage matrix during the course of OA, since the cartilage matrix pH is believed to be near neutral [9,10]. Here we report the patterns of expression of MMP-1, MMP-2, MMP-3 and MMP-9 by fibroblasts in subchondral defects from patients with OA of the hip.

Patients and methods

Patients

Total hip replacement was performed on 85 patients with OA of the hip between 1994 and 1996 at our institution. Samples of fibrous connective tissue within subchondral cystic defects were obtained from 78 of these patients, including 6 men and 72 women. The mean age of the women was 61 (39–83) years, while that of the men was 62 (45–72) years. Twenty-nine patients had bilateral OA and 49 patients had unilateral OA. Duration of symptoms varied from 3 to 195 months. Clinical assessment of the hip joint was based on the hip rating scale adopted by the Japanese Orthopaedic Association [6]. This system assigns a maximum of 100 points with the following maximum scores: pain 40, mobility 20, walking ability 20, and activities of daily living 20 points. The osteoblastic response to OA of the hip was classified radiographically into three types based on osteophyte formation, i.e., hypertrophic (Fig. 1A), normotrophic (Fig. 1B) or atrophic (Fig. 1C) [1].

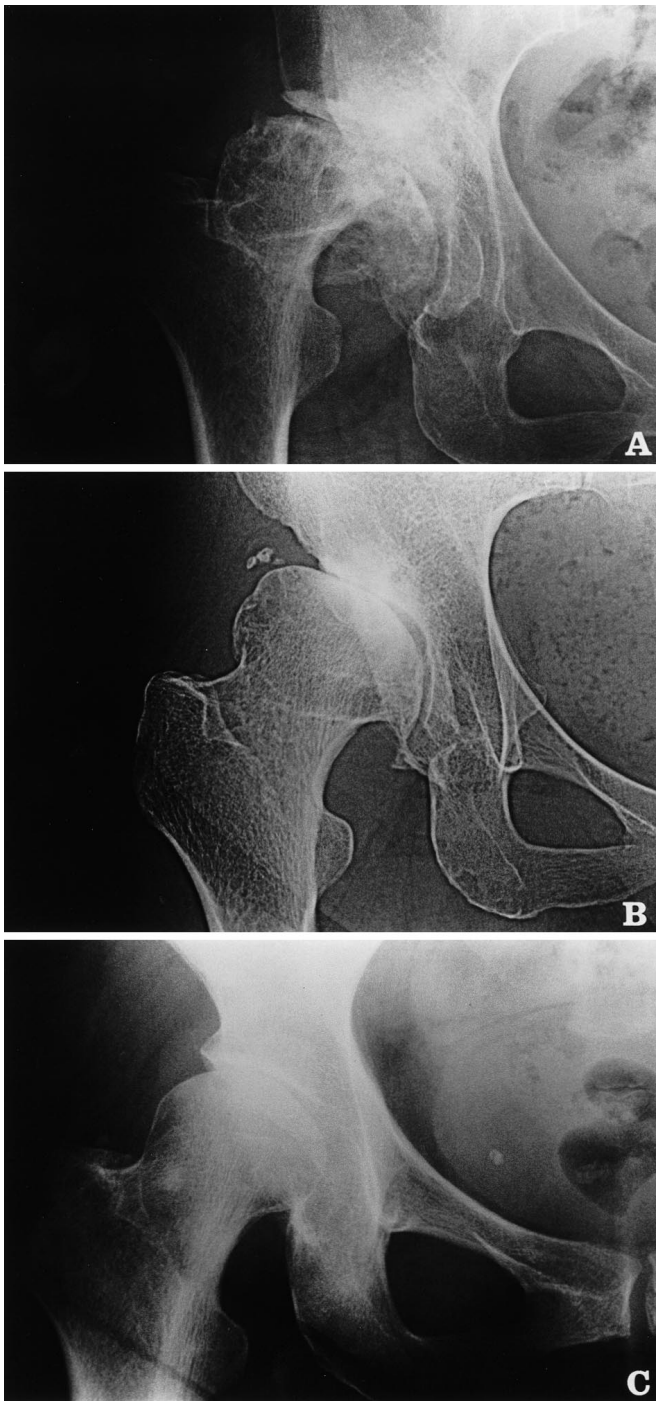


Fig. 1A–C *Roentgenographic classification of osteoblastic response in OA of the hip*. **A** Hypertrophic type with marked osteophytes and a large capital drop. **B** Normotrophic type with moderate osteophyte formation. **C** Atrophic type with little osteophyte formation

Cell culture

Fibrous connective tissue from subchondral cystic defects at weight-bearing sites of the acetabulum was obtained during operation. The tissue was cut into small fragments and incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. When fibroblasts had migrated sufficiently from the explants, they were subcultured with typsinization.

Western blot analysis

When fibroblasts reached confluence in small flasks (25 cm^2), the medium was replaced by 2 ml of fresh serum-free medium. The medium was collected after incubation for 3 days and used in the immunoblotting analyses. For immunoblotting, proteins of the culture medium (10 µl from 1×10^6 cells) separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions were transferred onto a nitrocellulose membrane. The membrane was blocked and treated for 1 h at room temperature with antibodies against human MMPs (anti-human-proMMP-1, proMMP-2, proMMP-3 and proMMP-9) [10,15]. The rest of the samples were stored at –80° for further Western blotting as controls. After extensive washing with phosphate-buffered saline (PBS) (pH 7.4), the membrane was incubated in alkaline phosphatase-conjugated rabbit IgG against sheep IgG. Immunoreactive MMPs were visualized using 5-bromo-4-chloro-3-indoyl phosphate and nitro blue tetrazolium.

Gelatin zymography

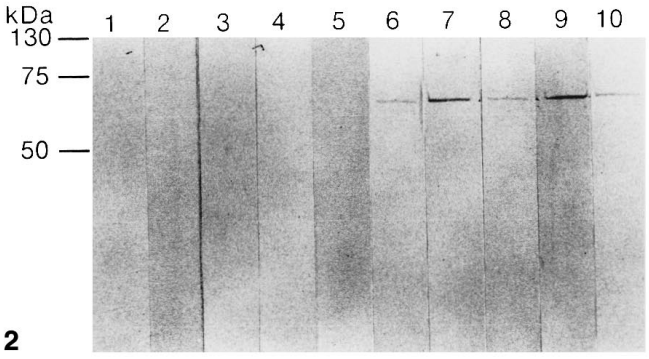
The same medium as used for Western blotting was analysed on a 10% SDS-PAGE containing 0.8 mg/ml of gelatin under reducing conditions as previously described [5]. Gels were washed in 1% Triton X-100 for 30 min and then incubated for 16 h at 37° in 50 mmol/l Tris buffer, pH 7.5, containing 0.15 mol/l NaCl and 5 mmol/l $CaCl_2$ or a buffer solution to which 10 mmol/l EDTA was added to inhibit MMP activity. Gels were stained with Coomassie Brilliant Blue R-250.

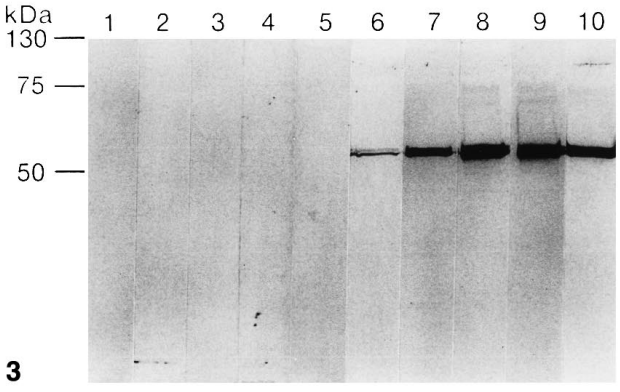
Results

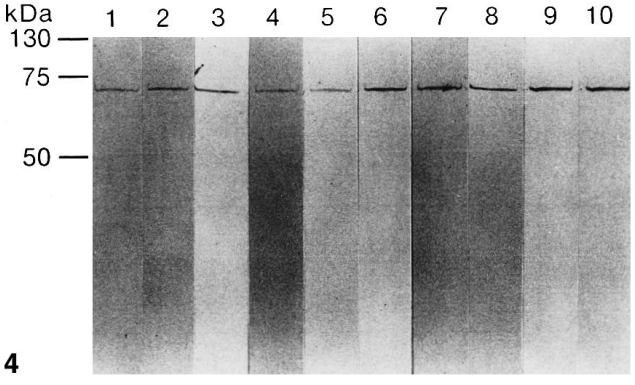
In all OA cases, fibroblasts began to migrate from the explants after incubation for at least 1 week and reached confluency ($0.93\sim1.01\times10^6$ cells/case) at 2–3 weeks.. Production of proMMP-1 and proMMP-3 was detected for 25 patients (Figs. 2, 3). There were two more cases that had very faint bands identical to MMP-1 and another three cases that had a small faint band identical to MMP-3 in subset 1. Synthesis of proMMP-2 was demonstrated for all OA patients (Fig. 4). However, the amount of proMMP-2 appeared to be constitutively high in 25 patients, which was confirmed by determination of the gelatinolytic activities of the samples (Fig. 5). Furthermore, fibroblasts from 10 patients also secreted MMP-9 (Fig. 6).

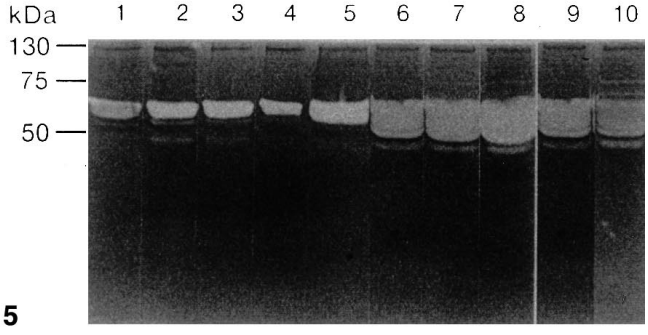
Based on production of MMPs within the hip joint, OA of the hip could be classified into three subtypes: subset 1, with only MMP-2 detectable, subset 2 with MMP-1 and MMP-3 detectable in addition to MMP-2, and subset 3 with MMP-1, 2, 3, and 9 all detectable. Significant differences in parameters among the three subsets were as follows:

1. Age: patients in subsets 2 and 3 were older than those in subset 1 ($P<0.01$, Mann-Whitney test). There was no significant difference between subsets 2 and 3 in age.
2. Sex: No significant difference was found among the three subsets for sex (chi-square test).
3. Unilateral or bilateral disease: Compared to patients in subset 1, those in subset 2 ($P<0.05$, chi-square test) and 3 ($P<0.02$) had mostly unilateral disease. There









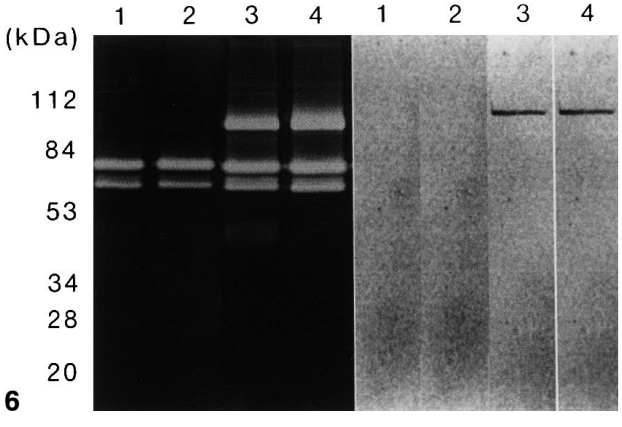


Fig. 2 Western blot detection of proMMP-1 in culture supernatant of fibroblasts (10 µl from 1×10^6 cells) from patients in subset 1 (*Lanes 1–5*), subset 2 (*Lanes 6 and 7*) and subset 3 (*Lanes 8–10*)

Fig. 3 Western blot detection of proMMP-3 in culture supernatant of fibroblasts (10 µl from 1×10^6 cells) from patients in subset 1 (*Lanes 1–5*), subset 2 (*Lanes 6 and 7*) and subset 3 (*Lanes 8–10*)

Fig. 4 Western blot detection of proMMP-2 in culture supernatant of fibroblasts (10 µl from 1×10^6 cells) from patients in subset 1 (*Lanes 1–5*), subset 2 (*Lanes 6 and 7*) and subset 3 (*Lanes 8–10*)

Fig. 5 Gelatin zymography of culture supernatant of fibroblasts (10 µl from 1×10^6 cells) from patients in subset 1 (*Lanes 1–5*), subset 2 (*Lanes 6 and 7*) and subset 3 (*Lanes 8–10*). Lanes 6–10 exhibit more gelatinolytic activity

Fig. 6 Comparison between fibroblasts in subset 1 (*Lanes 1 and 2*) and those in subset 3 (*Lanes 3 and 4*) for their production of pro MMP-9. Gelatin zymography (*left*) and immunoblotting (*right*) were done as described under materials and methods

was no significant difference between subsets 2 and 3 in laterality.

4. Duration of symptoms (months): Duration of symptoms was shorter in subset 3 than in subsets 1 ($P<0.01$, Mann-Whitney test) and 2 ($P<0.01$). It was also shorter in subset 2 than that in subset 1 ($P<0.01$).
5. Hip rating scale: A significant difference between subsets 1 and 2 was found solely in activities of daily living, the score for which was lower in subset 2 ($P<0.05$, Mann-Whitney test). Pain ($P<0.01$), walking ability ($P<0.01$), activities of daily living ($P<0.01$), and total hip score ($P<0.01$) were lower in subset 3 than in subset 1. On the other hand, mobility was larger in subset 3 ($P<0.05$) than in subset 1. A significant difference was observed between subsets 2 and 3 only in total hip score ($P<0.02$), which was lower in subset 3 than in subset 2.
6. Radiographic assessment: There was significantly more atrophic osteoblastic response in subsets 2 ($P<0.01$, chi-square test) and 3 ($P<0.01$) than in subset 1. No significant difference was found between subsets 2 and 3.

Discussion

The results of Western blotting and gelatin zymography in the present study suggest that there are at least three subtypes of OA of the hip differentiated by production of MMPs in the affected joint. One of the most prominent differences was in duration of clinical symptoms, which

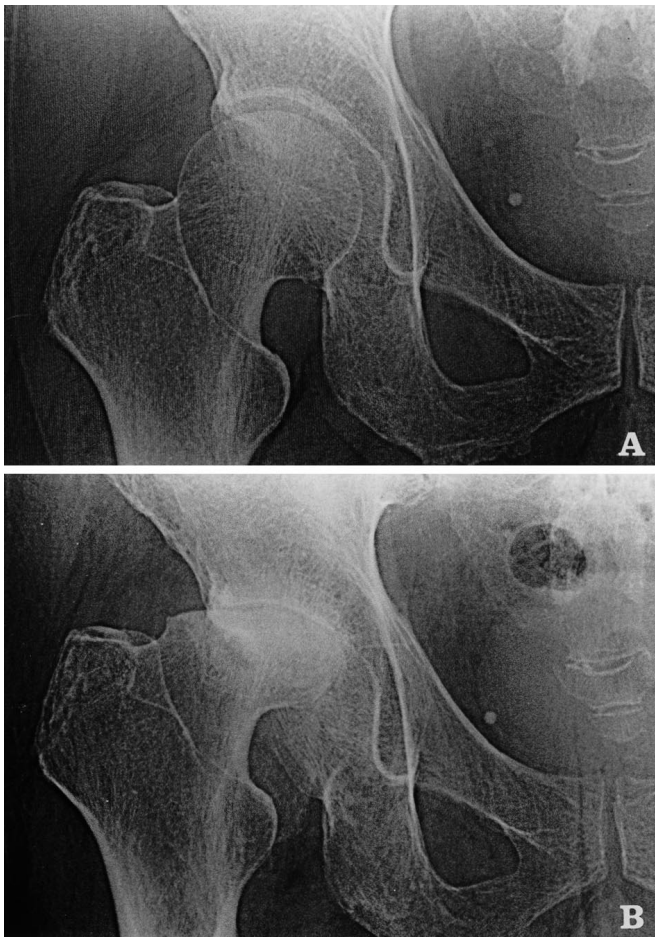


Fig. 7A,B *A 73-year-old woman* (Case 72). **A** A roentgenogram taken in Feburuary,1995 showed mild joint space narrowing. **B** A roentgenogram taken in June,1995. In the short term, both of acetabulum and the femoral head were markedly damaged

was shorter in subset 2 than in subset 1 and least in subset 3. The three biochemically different subtypes we isolated can therefore be referred to as the slowly progressive subset corresponding to subset 1, the moderately progressive subset corresponding to subset 2, and the rapidly progressive subset corresponding to subset 3.

MMP-2 (gelatinase A) can degrade denatured collagen as well as gelatin, fibronectin and elastin. Although this proteinase was detected in all subtypes by Western blot analysis, gelatinolytic activity was higher in the rapidly progressive subset than in the slowly progressive subset on zymographic testing. This difference in gelatinolytic activity may be related to the more rapid course of destruction in subset 3. MMP-1 (collagenase) and MMP-3 (stromelysin) are thought to play essential roles in degrading the matrix of bone and cartilage, respectively [3,10]. The larger amounts of MMP-1 and MMP-3 observed in the moderately and rapidly progressive subsets could be required for degeneration of bone and cartilage matrix and rapid scavenging of degradation product. MMP-9 has been implicated in the cellular migration and invasion in conditions such as inflammation, tumor invasion and metastasis [7]. In addition, recent studies of this proteinase in skeleton suggest that MMP-9 can digest cartilage proteoglycans and also play a role in osteoclastic bone resorption [12,17]. In the present study, MMP-9 was found only in the rapidly progressive subset. Production of MMP-9 might play a role in rapid invasion of fibrous connective tissue leading to massive destruction of osteoarthritic joint without compensatory bone formation.

Clinical characteristics of the rapidly progressive subset appear to coincide with those of a peculiar subset of OA previously termed rapidly destructive osteoarthritis (RDO) [11,13]. Six of 10 patients in the rapidly progressive subset had previous roentgenograms revealing minimal joint space narrowing 3–10 months prior to the first visit to our outpatient clinic (Fig. 7). Only one biochemical study of RDO is available, in which interleukin (IL)-1 stimulated synovial cells from patients with RDO produced significant amounts of MMP-1, 2 and 3 [8]. In our study, fibroblasts in the rapidly progressive subset generated detectable amounts of MMP-1, 2, 3 and 9 without stimulation by IL-1. Furthermore, fibroblasts in the slowly progressive subset secreted significant amounts of MMPs following stimulation with 10 ng/ml of IL-1 (data not shown). This finding suggests that fibroblasts in the rapidly progressive subset might be upregulated under specific conditions.

Recent advances in enzyme immunoassay using monoclonal antibodies have enabled quantitative analysis of MMPs [4,18]. Elevated serum levels of MMP-3 have been observed in patients with rheumatoid arthritis and generalized OA [14]. It is possible that large amounts of MMPs are produced by fibroblasts in some subsets of OA of the hip and released into serum via bone marrow microcirculation. In addition, chondrocytes in articular cartilage and bone cells in subchondral bone have been reported to produce MMPs. When these cells undergo rapid degeneration, MMPs might be released and contribute to an increase in concentration of MMPs in the hip joint. Thus, detection of elevated serum levels of MMPs could be of diagnostic significance in order to identify the rapidly progressive subset. Further attempts to quantitate concentrations of MMPs in serum as well as in culture medium are clearly needed to obtain a more precise biochemical classification of OA of the hip.

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