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PRELIMINARY REPORT

Relationship Between Osteoprotegerin/Osteoclastogenesis Inhibitory Factor Concentration in Synovial Fluid and Disease Severity in Individuals With Osteoarthritis of the Knee

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We studied the relationship between osteoprotegerin (OPG)/osteoclastogenesis inhibitory factor (OCIF) concentration in synovial fluid from individuals with osteoarthritis (OA) of the knee and the severity of this condition. The study population included 111 Japanese women with knee OA (153 knees) and 23 normal controls. Osteoarthritic changes were graded according to the system of Kellgren and Lawrence. The concentration of OPG/OCIF in synovial fluid increased with severity of knee OA and was significantly higher in individuals with OA of grade IV than in those with OA of grade 0 or grade I. It has been shown in a previous study that administration of OPG/OCIF prevents cartilage destruction in adjuvant-induced arthritis in rats. The increase in the concentration OPG/OCIF in synovial fluid of individuals with knee OA might thus reflect a compensatory response to degeneration of articular cartilage and serve to protect cartilage rather than be a cause of OA.

OSTEOPROTEGERIN (OPG)/osteoclastogenesis inhibitory factor (OCIF) inhibits osteoclastogenesis by interrupting intercellular signaling between osteoclast progenitors and bone marrow stromal cells.[1,2] We previously showed that the serum concentration of OPG/OCIF increases with age in healthy men and women and is higher in postmenopausal women with osteoporosis than in age-matched normal controls.[3] A number of lines of evidence indicated that OPG/OCIF inhibits bone resorption in vivo.[1] In addition, administration of OPG/OCIF prevented cartilage destruction in adjuvant-induced arthritis in rats.[4] However, it is unknown whether this factor plays a role in the pathogenesis of osteoarthritis (OA) of the knee. With the use of an enzyme-linked immunosorbent assay (ELISA) that we developed,[3] we have now measured the OPG/OCIF concentration in synovial fluid from individuals with OA of the knee and examined the relationship between the concentration of this cytokine and the severity of OA.

SUBJECTS AND METHODS

The study population included 111 unrelated Japanese women with knee OA (153 knees) and 23 normal controls. The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital, and informed consent was obtained from each subject. Anteroposterior radiographs of weight-bearing knees were obtained, and osteoarthritic changes were graded by 3 orthopedic surgeons according to the system of Kellgren and Lawrence[5]: 0, no osteophytes or joint-space narrowing; I, questionable presence of either osteophytes or joint-space narrowing, or both; II, either definite presence of osteophytes with possible joint-space narrowing or definite mild joint-space narrowing with or without osteophytes; III, definite moderate joint-space narrowing (≥50%) (cysts or sclerosis may be present, and osteophytes are usually present); and IV, severe joint-space narrowing. No significant interobserver variability in grading OA was detected (κ coefficient, 0.62). Lumbar spine (L2-L4) and total body bone mineral density (BMD) was measured by dual-energy x-ray absorptiometry.

Synovial fluid in the affected knee joint of individuals with OA was aspirated, centrifuged at 3,500 × *g* for 10 minutes at 4°C to remove cells and debris, and stored at −30°C. Blood samples were centrifuged at 1,600 × *g* for 15 minutes at 4°C, and serum was separated and stored

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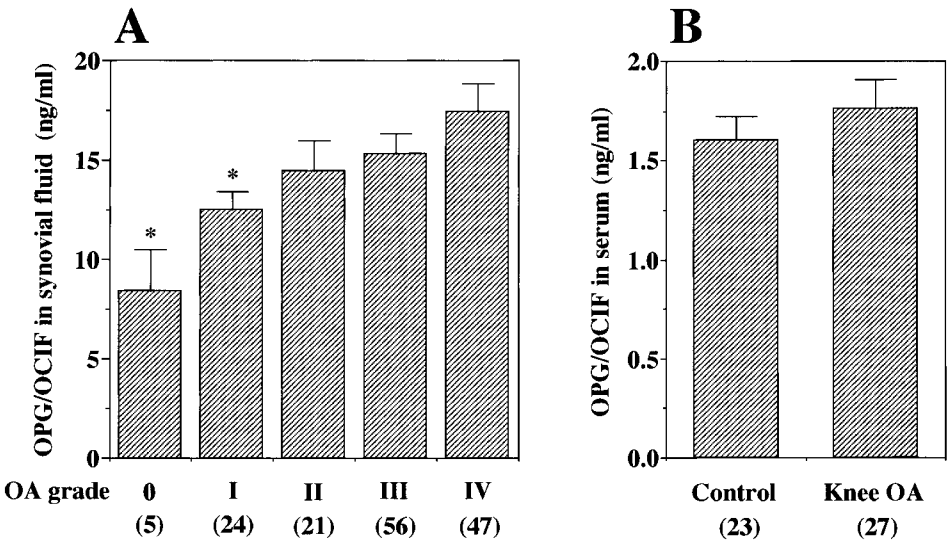

Fig 1. (A) Concentration of OPG/OCIF (means ± SE) in synovial fluid from individuals with OA of the knee according to the classification of Kellgren and Lawrence. *$P = .01$ *v* grade IV. (B) Serum concentrations of OPG/OCIF (means ± SE) in controls and individuals with knee OA. There was no significant difference in age between control (mean ± SD, 64.3 ± 9.2 years) and knee OA (64.3 ± 8.1 years) groups. The number of subjects is indicated in parentheses.

at −30°C. The concentrations of OPG/OCIF in synovial fluid and serum were determined by ELISA as previously described.[3] The minimum detection limit of this assay system was 32.5 pg/mL.

Data were compared among subjects with OA of the knee of different severity by 1-way analysis of variance and Fisher's projected least significant difference test. Unpaired Student's *t* test was used for comparisons between the control and patient groups.

RESULTS

The concentration of OPG/OCIF in synovial fluid increased with severity of knee OA (Fig 1A) and was significantly higher in individuals with knee OA of grade IV than in those with knee OA of grade 0 or grade I. The concentration of OPG/OCIF in serum was lower than that in synovial fluid and did not differ significantly between individuals with knee OA and age-matched normal controls (Fig 1B), indicating that the increase in the concentration of OPG/OCIF in the synovial fluid of patients with knee OA was not systemic, but rather, localized to the joint. Neither L2-L4 nor total body BMD differed significantly between patient and control groups (data not shown).

DISCUSSION

Kong et al[4] showed that OPG ligand (OPGL)/osteoclast differentiation factor (ODF) expressed in activated T cells is a key regulator of joint destruction and bone loss in adjuvant-induced arthritis in rats, and that inhibition of OPGL/ODF activity by administration of OPG/OCIF prevented cartilage destruction and preserved the integrity of cartilage. Adjuvant-induced arthritis mimics pathologic features of human rheumatoid arthritis, which apparently differs from those of OA. However, these investigators also detected the expression of OPGL/ODF in T cells isolated from the joints of humans with OA, suggesting that OPG/OCIF may also play a role in the pathophysiology of OA.[4] Synovial fibroblasts of individuals with rheumatoid arthritis released OPG/OCIF in vitro.[6] OPG/OCIF mRNA is abundant in cells of the mouse chondrogenic line ATDC (Sasaki A, personal communication, January 2000). These observations suggest that synovial fibroblasts and articular chondrocytes may be the sources of OPG/OCIF production in joints, although the role of the latter remains to be confirmed. Given that OA is characterized by progressive deterioration of the cartilage of diarthrodial joints, the increase in the concentration of OPG/OCIF in synovial fluid of individuals with knee OA might reflect a compensatory response by chondrocytes or synovial fibroblasts to destabilization of the coupling between the degradation and synthesis of articular cartilage. The increased concentration of OPG/OCIF might thus serve to protect cartilage rather than be a cause of OA. However, the balance between OPG/OCIF and OPGL/ODF may be an important determinant of bone resorption and cartilage deterioration. It is possible that both molecules are increased in osteoarthritic joints. It is also possible that the expression of OPGL/ODF is more abundant than that of OPG/OCIF, which tips the balance in favor of bone resorption and cartilage deterioration.

REFERENCES

1. Simonet WS, Lacey DL, Dunstan CR, et al: Osteoprotegerin: A novel secreted protein involved in the regulation of bone density. Cell 89:309-319, 1997

2. Tsuda E, Goto M, Mochizuki S, et al: Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 234:137-142, 1997

3. Yano K, Tsuda E, Washida N, et al: Immunological characterization of circulating osteoprotegerin/osteoclastogenesis inhibitory factor: Increased serum concentrations in postmenopausal women with osteoporosis. J Bone Miner Res 14:518-527, 1999

4. Kong Y-Y, Feige U, Sarosi I, et al: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304-309, 1999

5. Kellgren JH, Lawrence JS: Atlas of Standard Radiographs, vol 2. The Epidemiology of Chronic Rheumatism. Oxford, UK, Blackwell Scientific, 1963

6. Takayanagi H, Iizuka H, Juji T, et al: Involvement of receptor activator of nuclear factor κB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis Rheum 43:259-269, 2000