Abnormal osteocalcin binding in rheumatoid arthritis

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Abstract

Studies of osteocalcin in the serum and synovial fluid of patients with rheumatoid arthritis (RA) and osteoarthritis (OA) showed the presence of significant amounts of osteocalcin in synovial fluid and that the values in RA synovial fluid were significantly lower than in OA synovial fluid. In addition, the osteocalcin in OA synovial fluid bound almost completely to hydroxyapatite whereas a significant proportion of the osteocalcin in RA synovial fluid did not. These studies suggest that patients with severe RA produce low amounts of active osteocalcin and higher than expected amounts of inactive osteocalcin in the synovial fluid. They provide some evidence that osteoblast function may be abnormal in the osteoporosis of RA.

Osteoporosis is a common clinical problem in patients with rheumatoid arthritis (RA). The exact pathogenesis of this type of bone disease is unknown, but one possible explanation is an abnormality of osteoblast function or secretion. Osteocalcin (bone γ-carboxyglutamic acid (Gla) protein) is a vitamin K dependent protein, which is a specific product of the osteoblast.1

The osteocalcin found in serum comes from new cellular synthesis rather than from the release of bone matrix protein during bone resorption2 and it contains three residues of glutamic acid, which are carboxylated to form carboxyglutamic acid. This carboxylated form of osteocalcin is thought to be the active form of the protein as it binds strongly to hydroxyapatite.3

Studies of bone status in patients with RA have shown varying results for osteocalcin measurements. Several studies have shown reduced mean serum osteocalcin values in RA,4 5 suggesting reduced bone formation. Another study found no relation between serum osteocalcin and the inflammatory activity of the arthritis,6 and a further report described increased values of serum osteocalcin in RA, suggesting increased bone turnover.7 These different results may reflect heterogeneity of bone involvement, the effect of different types of treatment, or methodological differences in the osteocalcin assay.

The radioimmunoassays at present in use for measurement of osteocalcin do not distinguish between the carboxylated (active) and non-carboxylated (inactive) forms of osteocalcin. It is therefore unknown whether the reports mentioned above are describing the fully carboxylated form or otherwise. The two forms of osteocalcin may be distinguished as carboxylated osteocalcin binds to hydroxyapatite whereas non-carboxylated osteocalcin does not.2 We therefore determined the total and non-hydroxyapatite-bound osteocalcin in the serum and synovial fluid of patients with RA and in patients with osteoarthritis (OA) for comparison.

Patients and methods

Paired serum and synovial fluid samples were collected from 11 patients with RA and 13 patients with OA attending the outpatient rheumatology clinic for therapeutic knee aspiration. The samples were stored at −20°C before assay for osteocalcin and C reactive protein. Osteocalcin was measured by radioimmunoassay (CIS (UK), Wycombe, Bucks) and C reactive protein by LC-Partigen immunodiffusion plates (Behring Diagnostics, Hounslow, Middlesex).

Binding of osteocalcin to hydroxyapatite was determined in nine pairs of samples from patients with OA and in 10 sample pairs from patients with RA, as described by Price et al.2

Serum or synovial fluid for osteocalcin measurement was divided into two portions. One aliquot was extracted with hydroxyapatite (20 mg to 200 μl serum or synovial fluid), the mixture vortexed, turned end over end at 4°C for 30 minutes, and centrifuged for five minutes in a table top clinical centrifuge. The resulting supernatant containing non-hydroxyapatite-bound osteocalcin and the other aliquot containing the total amount of osteocalcin were assayed for osteocalcin in the same radioimmunoassay.

The hydroxyapatite bound osteocalcin was obtained by subtraction, and the lower limit of sensitivity for osteocalcin was 0.35 ng/ml.

Results

Significant amounts of total osteocalcin were detected in the synovial fluid, and dilution of synovial fluid samples showed linearity when the osteocalcin values were compared with those in serum. The serum total osteocalcin values in RA and OA were normal (5.8 (SD 2.08) and 6.5 (2.5) ng/ml respectively), whereas the values in RA synovial fluid were significantly lower than those in OA synovial fluid (3.24 (2.05) and 5.1 (2.1) ng/ml respectively, p<0.04).

Up to 25% of the osteocalcin in normal, OA, and RA serum did not bind to hydroxyapatite. The osteocalcin in OA synovial fluid, however, bound almost completely to hydroxyapatite (0–1.9% unbound), whereas a significant proportion of the osteocalcin in RA synovial fluid
HA=hydroxyapatite.
SF=synovial fluid; RA=rheumatoid arthritis; OA=osteoarthritis; and non-

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(0-92% unbound) did not. The carboxylated
form of osteocalcin, therefore, was present in
similar concentrations in OA synovial fluid and
serum. The RA synovial fluid osteocalcin, on
the other hand, was present at a concentration
about half that in serum, and in some patients
with RA the synovial fluid contained significant
amounts of osteocalcin that were not fully
carboxylated (inactive) (figure). In addition, RA
synovial fluid contained measurable amounts
of C reactive protein, whereas OA synovial fluid
did not. High C reactive protein values were
found in samples of synovial fluid containing
high amounts of non-hydroxyapatite-bound
osteocalcin (r=0.77, p<0.01).

Discussion
To our knowledge this is the first report of
hydroxyapatite binding studies on the osteocalcin
found in synovial fluid. The interpretation of
synovial fluid values in arthritis is difficult
because normal synovial fluid is not freely
available for comparison. Previous work from
our laboratory has shown that substances such
as 25-hydroxyvitamin D and retinol are present
in synovial fluid at approximately half the
concentration of that in serum in both RA and
OA.8 9 9 Thus the RA synovial fluid values for
total osteocalcin we obtained are of the order
expected, whereas the values obtained in OA
synovial fluid are higher than expected. The
latter may reflect new bone formation within the
joint.

The values of osteocalcin obtained after
hydroxyapatite binding in RA synovial fluid are
particularly interesting as they suggest that
patients with severe RA produce low amounts
of active osteocalcin and higher than expected

amounts of inactive osteocalcin in their synovial
fluid. The origin of synovial fluid osteocalcin is
not clear. It may be derived from the circulation
and thus represent new synthesis from osteo-
blasts, or possibly from break down of bone
surrounding the joint. The relation between C
reactive protein and the non-hydroxyapatite-
bound osteocalcin suggests, however, that the
rheumatoid process may interfere with normal
binding of osteocalcin to bone, and contribute
to the pathogenesis of osteoporosis in RA.

Another possible explanation may be defective
vitamin K activity as low concentrations of
serum vitamin K have been found in patients
with osteoporosis.10 Osteocalcin is vitamin K
dependent and patients receiving vitamin K
antagonists, such as phenprocoumon, have
higher than normal amounts of inactive osteo-
calcin in their serum.11 As the serum osteocalcin
in our patients showed normal hydroxyapatite-
binding it is unlikely that the patients were
deficient in vitamin K. In view of the higher
amounts of non-hydroxyapatite-bound osteo-
calcin in RA synovial fluid than in OA synovial
fluid, however, it is possible that synovial
vitamin K activity is defective in patients with
RA.

A further possibility relates to synovial fibro-
blast metabolism. The cells that comprise the
pannus in RA are also exposed to local hormones
or hormones carried to the site by the circula-
tion—for example, parathyroid hormone. Para-
thyroid hormone has its primary action on
target cells in bone—for example, osteoblasts,
but it also stimulates the production of cAMP
from synovial cells in culture. As parathyroid
hormone stimulates bone resorption, increased
sensitivity of synovial fibroblasts to its effects
might be an additional factor producing connec-
tive tissue destruction and subsequent release
of osteocalcin into the joint.12

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