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Changes in systemic levels of insulin-like growth factors and their binding proteins in patients with rheumatoid arthritis

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ABSTRACT

Objective

To determine whether circulating levels of insulin-like growth factors and their binding proteins are altered in patients with adult onset rheumatoid arthritis.

Methods

Plasma-levels of insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding-protein 2 (IGFBP-2), and IGFBP-3 were measured by radioimmunoassay in 53 patients with clinically active rheumatoid arthritis (RA) and in 51 control subjects.

Results

In RA patients plasma levels of IGF-II were lower (601 ± 34 vs. 731 ± 32 µg/L (mean ± SEM); p = 0.005; Mann-Whitney rank sum test) than in age- and sex-matched controls (n = 30 per group). In contrast, plasma levels of IGFBP-2 (412 ± 40 vs. 254 ± 20 µg/L; p = 0.003) and IGFBP-3 were elevated in RA patients (3.34 ± 0.19 vs. 2.87 ± 0.21 mg/ L; p = 0.019) as compared with the matched controls.

The molar ratio of IGF-I to IGFBP-3 was significantly reduced in subjects with RA (0.18 ± 0.01 vs. 0.24 ± 0.02; p = 0.008). Furthermore, in RA patients plasma levels of IGFBP-2 were positively (r = 0.45), and levels of IGF-2 negatively (r = -0.45) correlated with circulating levels of C-reactive protein (p < 0.01 in both cases; Spearman rank correlation).

Conclusion

Increased levels of IGFBPs in RA may result in the reduced availability of free IGFs that can bind to IGF receptors. The observed changes in the IGF system may thus participate in the catabolic processes in rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a systemic disease characterized by chronic joint inflammation, eventually leading to destruction of articular cartilage, tendons, ligaments, and periarticular bone. The insulin-like growth factors, IGF-I and IGF-II, represent a family of growth hormone dependent proteins involved in the regulation of longitudinal growth as well as in the maintenance of articular cartilage and bone homeostasis. IGF-I and, to a lesser extent, IGF-II promote chondrocyte proliferation and enhance the synthesis of collagen and proteoglycans by chondrocytes (1-4). Furthermore, IGF-I can inhibit cytokine-mediated cartilage degradation *in vitro* by antagonizing the catabolic effects of IL-1 and TNFa (5, 6).

The bioactivity of IGFs is regulated by IGF binding proteins (IGFBPs), of which IGFBP-3 is the most abundant form in human plasma, followed by IGFBP-2 (7). Since IGFs can participate in the regulation of bone and cartilage metabolism, there has been considerable interest in their role in joint diseases. The results communicated so far are, however, inconsistent. Plasma levels of IGF-I were reportedly reduced in children with juvenile chronic arthritis (8, 9). In patients with RA both normal and reduced serum levels of IGF-I have been communicated (10-12). Studies of patients with osteoarthritis have failed to yield substantial evidence of systemic alterations in IGF levels in these subjects (13, 14).

The present study was therefore undertaken to study circulating levels of IGFs and their binding proteins in patients with active RA.

Patients and methods

Plasma samples were obtained from 53 patients (39F; 14M) with rheumatoid arthritis meeting the American College of Rheumatologists diagnostic criteria for RA (15), and from 51 control subjects (21F; 30 M) without evidence of systemic inflammatory joint disease. Controls were either healthy volunteers or out-patients seen for minor surgical problems unrelated to joint pathology. The RA patients all had active RA with at least 3 swollen and tender joints. Informed consent was obtained in all cases. In the RA group the mean age (range) was 59 (32-80) years. In the control group the mean age (range) was 51 (18-80) years. Plasma was immediately separated from cells by centrifugation (3,000 xg for 10 min), and stored in aliquots in polypropylene vials at -20°C until use.

IGF-I in plasma was measured with a specific radioimmunoassay (RIA; Mediagnost,Tübingen,Germany). This as-

Table I. Concentrations of insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) in the plasma of 53 patients with rheumatoid arthritis (RA), and 51 control subjects. The IGF-I/IGFBP-3 ratio reflects the molar ratio. Data represent means (SEM) of the number of observations indicated. P-values are based on the Mann-Whitney rank sum test.

	Control	RA	P
IGF-I (µg/L)	134 (7)	100 (8)	< 0.001
IGF-II (µg/L)	721 (22)	647 (28)	0.019
IGFBP-2 (µg/L)	290 (44)	442 (58)	< 0.001
IGFBP-3 (mg/L)	2.77 (0.16)	3.22 (0.15)	0.007
IGF-I/IGFBP-3 (ratio)	0.27 (0.02)	0.17 (0.01)	< 0.001

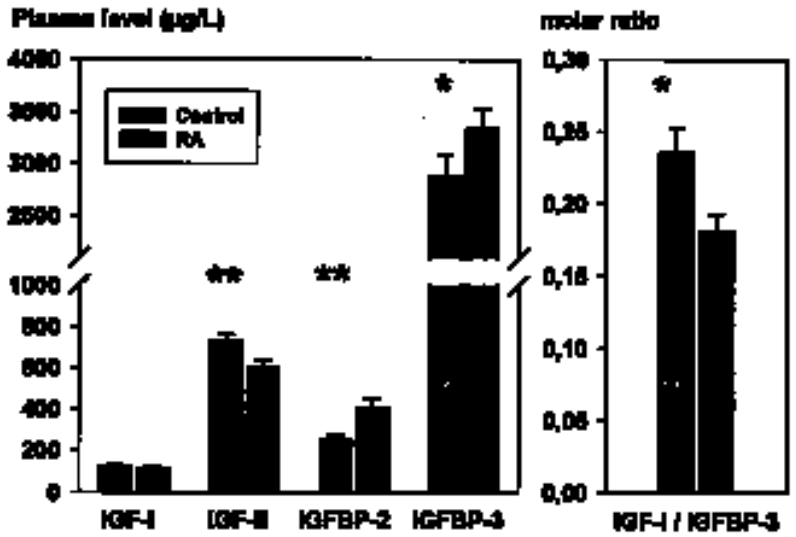

Fig. 1. Plasma concentrations of insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) in 30 patients with rheumatoid arthritis (RA), and 30 control-subjects matched for age and sex. The IGF-I/IGFBP-3 ratio reflects the molar ratio. Data represent means and SEM of the number of subjects indicated. ($p < 0.02$; **$p < 0.01$ versus the respective RA group (Mann-Whitney rank sum test).

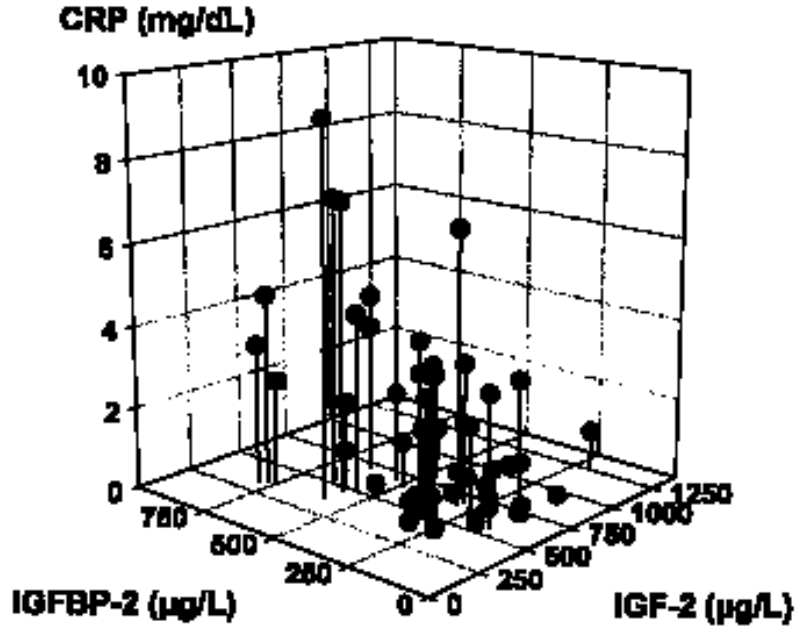

Fig. 2. Plasma concentrations of insulin-like growth factor II (IGF-II),IGF-binding protein-2 (IGFBP-2), and C-reactive protein (CRP) in 53 patients with rheumatoid arthritis (RA). Plasma levels of IGFBP-2 were positively ($r = 0.45$), and levels of IGF-2 were negatively ($r = -0.45$) correlated with CRP-levels ($p < 0.01$ in both cases; Spearman rank correlation).

say uses an excess of IGF-II to eliminate interference by IGFBPs (16).

IGF-II was determined using a specific RIA (17). In this assay, an excess of IGF-I is used to eliminate interference by IGFBPs.

IGFBP-2 was assayed in plasma using a specific RIA as described elsewhere (18), with minor modifications. Recombinant hIGFBP-2 (a gift of Sandoz, Basel, Switzerland) was used as a standard and for the tracer preparation instead of the originally used synthetic peptide hIGFBP-2(176-190). Plasma was diluted 1:20 with assay buffer before measurement.

IGFBP-3 was measured with a specific RIA kit from Mediagnost. This assay does not cross-react with IGFBP-1 or IGFBP-2.

The standard curves paralleled the sample dilution curves in all cases.

Concentrations of C-reactive protein were determined using a Boehringer nephelometer.

Results

IGF-I, IGF-II, IGFBP-2, and IGFBP-3 were detectable in all the samples assayed. In the RA group, plasma concentrations of IGF-I and IGF-II were reduced, and levels of IGFBP-2 and IGFBP-3 were elevated, as compared with the controls (Table I).

Since the age ($p < 0.05$, t-test) and sex ratio ($p < 0.05$, c^2 test) differed between RA patients and controls, it was necessary to match both groups for age and sex ($n = 30$ per group). Between the matched groups, the difference in the mean IGF-I plasma levels was no longer significant [113 ± 10 mg/L in the RA group, and 122 ± 8 mg/L in the control group (mean ± SEM)], whereas the plasma concentrations of IGF-II, IGFBP-2, and IGFBP-3 still differed between RA patients and controls (Fig. 1; the respective numerical data are reproduced in the abstract).

The molar ratio of IGF-I to IGFBP-3 was significantly reduced in subjects with RA (0.18 ± 0.01 vs. 0.24 ± 0.02; $p = 0.008$,Mann-Whitney rank sum test).

Furthermore, in RA patients the plasma levels of IGFBP-2 were positively ($r = 0.45$), and the levels of IGF-2 were negatively ($r = -0.45$) correlated with

circulating levels of C-reactive protein ($p < 0.01$ in both cases; Spearman rank correlation; Fig. 2).

Discussion

An equilibrium between matrix synthesis and matrix degradation is necessary to maintain the homeostasis of articular cartilage and bone. This balance is disturbed in rheumatoid arthritis, where an excess of catabolic cytokines participates in the upregulation of matrix-degrading proteinases while matrix synthesis of cartilage and bone is simultaneously inhibited. These processes result in demineralization and the erosion of bone, particularly near joints, and in the destruction of articular cartilage.

Insulin-like growth factor I is the major anabolic factor in human serum and synovial fluid with respect to the proteoglycan synthesis of chondrocytes exposed to these fluids (19). In arthritis, however, chondrocytes seem to be unresponsive to IGF-I (20), a fact that may relate to the modulation of IGF-I-bioactivity by increased levels of IGF binding proteins (21). IGFs also have important anabolic effects on osteoblasts, and IGF deficiency has been implicated as a risk factor for developing osteoporosis (22).

We found increased systemic levels of IGFBP-2 and IGFBP-3 in patients with RA. At the same time, IGF-II plasma levels were reduced in RA patients, and there was a non-significant tendency towards lower IGF-I plasma concentrations in these subjects. These divergent findings for IGFs and their binding proteins are in accordance with recently reported data in rodents. Induction of adjuvant arthritis in rats led to decreased growth hormone (GH) secretion, increased systemic levels of IGFBP-3, and reduced concentrations of IGF-I both in serum and in the liver (23).

Johansson *et al*. (12) studied 13 middle-aged women with RA and compared them with 15 female controls of similar age. As in our study, the effects of sex and age were thus accounted for. In the RA group, they found lower serum-levels of IGF-II, which is in accordance with our matched pairs data. IGF-I plasma concentrations were also reduced in their RA patients while in our study there was only a non-significant tendency towards lower systemic IGF-I levels in the RA group when the groups were matched for age and sex. Johansson *et al*. (12) found no difference in IGFBP-3 between their 2 groups, possibly because of the small numbers of patients studied.

Fernihough *et al*. (11) examined 14 patients with RA (mean age 63; male/female ratio 1:6), and 11 normal controls (mean age 41; male/female ratio 8:3). Similar to our study, they found a non-significant tendency towards lower IGF-I serum levels, and significantly reduced serum concentrations of IGF-II in RA patients. In contrast to our findings, however, IGFBP-3 serum levels were reportedly lower in their RA group. At the same time, on the other hand, they reported a strong positive correlation between serum IGFBP-3 and disease activity, as assessed by serum CRP. This would indicate that patients with highly active RA have higher IGFBP-3 serum levels than those with only mildly active or inactive disease. Since IGFBP-3 levels tend to decrease with increasing age in adults, and their controls were much younger than their RA patients, the observed differences between the results of Fernihough *et al.* (11) and our data may well be explained by the confounding effect of age. Moreover, only patients with clinically active RA were included in our study.

As far as synovial fluid is concerned, IGFBP-3 levels have been consistently found to be elevated in RA (11, 24). Synovial fluid is largely a plasma filtrate; however, the local production of IGFBP-3, as well as the local rate of IGFBP-3 proteolysis can also influence the IGFBP-3 concentration in joint fluids.

Our data do not permit definite conclusions regarding the cause or the consequences of altered plasma levels of IGF and IGF binding proteins in RA patients. It has been shown that IL-1 and TNF inhibit IGF synthesis after GH stimulation in hepatocyte cultures (25, 26). Increased cytokine release in RA might thus inhibit IGF production in the liver. In RA the GH-IGF axis might, however, also be altered at the pituitary level, since GH reportedly shows a blunted response to GHRH stimulation in newly diagnosed, untreated RA patients (27). An increased breakdown of IGFs in chronic inflammation is also theoretically possible.

The observed increase in plasma IGFBPs may be due to increased cytokine levels in RA, since IL-1 and TNFa have been shown to induce IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-4 (28). Lipopolysaccharides, which induce IL-1, also increase circulating IGFBPs (29). Finally, IGFBP proteolysis seems to be decreased in chronic inflammation (11).

Possibly the most relevant finding among the data reported here, is the observed decreased IGF/ IGFBP ratio in the plasma of patients with RA. Similar changes have been described previously in RA synovial fluid (11). In conjunction with the other results reported here, a decreased IGF/ IGFBP ratio will likely result in the reduced availability of free IGFs that can bind to IGF receptors. Since IGFs are among the most important anabolic factors for the maintenance of matrix homeostasis of articular cartilage and bone, a shortage of IGFs could contribute to accelerated tissue breakdown in chronically inflamed joints. This is especially important in view of a possibly impaired IGF response of chondrocytes in an arthritic milieu (20, 21). The observed changes in the IGF system did in part parallel the disease activity. They may therefore be involved in the pathophysiologic process in RA.

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