INSULIN-LIKE GROWTH FACTOR-I AND INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 SERUM LEVELS IN ANKYLOSING SPONDYLITIS

E. TOUSSIROT, N. U. NGUYEN,* G. DUMOULIN,* J. REGNARD* and D. WENDLING

Department of Rheumatology and *Explorations Fonctionnelles Rénales, Météaboliques et Endocriniennes, University Hospital J.Minjoz, F-25030 Besançon, France

SUMMARY

Objective. Low bone mass, vertebral osteopenia and fractures have been described in patients with ankylosing spondylitis (AS), but the aetiology of this osteoporosis (OP) remains unknown. Insulin-like growth factor-I (IGF-I), a bone-promoting peptide, may be considered as reflecting osteoblast function as well as its main binding protein, insulin-like growth factor binding protein-3 (IGFBP-3). Both were found to be decreased in post-menopausal women and male patients with idiopathic OP. In this study, we aimed to measure the circulating IGF-I and IGFBP-3 in AS patients.

Methods. Thirty-three AS patients were compared to 23 healthy controls. Bone mineral density (dual X-ray absorptiometry) was measured at the spine and the femoral neck. We determined the serum levels of growth hormone (GH), insulin, glycaemia, and the IGF-I and IGFBP-3 serum concentrations.

Results. A lowered lumbar spine bone mineral density was found in the AS group (AS: 0.946 g/cm², controls: 1.02 g/cm²; \( P = 0.05 \)). AS patients had a higher glycaemia than controls, but results were in the normal range. There were no significant differences in the mean values for GH and insulin. Mean IGF-I serum levels were 218.3 ng/ml (± 72.4) in patients and 212.1 (± 71.1) in controls (\( P = 0.75 \)). The serum concentrations of IGFBP-3 were significantly lower in AS (3.29 ± 0.6 μg/ml) than in healthy subjects (3.63 ± 0.6 μg/ml; \( P = 0.05 \)). There was a negative correlation between the serum IGFBP-3 concentration and erythrocyte sedimentation rate (\( r = -0.39; P = 0.025 \)).

Conclusions. Since IGFBP-3 is an important cofactor for IGF-I and modulates its bioavailability and activity in bone, these data suggest that osteoblast cell function could be impaired in AS. Inflammation could play a role in this IGFBP-3/IGF-I axis involvement. However, further studies are warranted to determine the role of the other growth factors and their binding proteins in the OP of AS.

KEY WORDS: Insulin-like growth factor-I, Osteoporosis, Ankylosing spondylitis, Insulin-like growth factor binding proteins.

ANKYLOSING spondylitis (AS) is an inflammatory rheumatic disease with a main involvement of the spine and sacroiliac joints. Sacroiliac joint pain and backache are typical features of AS, and spine ankylosis is progressively induced by specific ossifications or syndesmophytes. Osteoporosis (OP) is a complicating feature of this condition [1] and there is some evidence of a certain degree of osteopenia in AS: established AS patients had a higher incidence of vertebral crush fractures [2, 3] and a decreased bone mineral density (BMD) at the spine and the femoral neck [4]. This lowered spine BMD was also observed in early AS patients without syndesmophyte formation [5]. The aetiology of this OP remains controversial; a reduced range of spinal movement in ankylosing patients, the treatments given or the inflammatory cytokines could contribute to this bone loss [1].

Insulin-like growth factor-I (IGF-I), or somatomedin C, is a bone-promoting peptide which mediates the effect of growth hormone (GH) at the tissue level, including bone [6, 7]. It has primarily marked anabolic actions on bone by increasing collagen gene expression and collagen synthesis, and inducing preosteoblast differentiation and proliferation. Thus, IGF-I promotes bone formation. It is produced by various tissues and has autocrine and paracrine actions. The circulating IGF-I is presumed to be mainly derived from liver, but is considered to exert endocrine actions. IGF-I is regulated by GH itself and also by its binding proteins (IGFBPs) [6]. These proteins can modulate the bioavailability of IGF-I. There are currently six or seven known circulating IGFBPs and they are all synthesized by osteoblasts. IGFBP-3 is the predominant protein that is linked to IGF-I.

It was recently reported that post-menopausal women [8] and male idiopathic osteoporotic patients [9] had decreased serum levels of IGF-I and IGFBP-3. It was thought that these lower IGF-I and IGFBP-3 levels reflected impaired osteoblastic function. Serum markers of bone turnover have been assessed in AS in order to explain the OP of this condition. Serum calcium, phosphorus and alkaline phosphatases were found to be normal, as well as osteocalcin (OC) [10, 11]. On the contrary, urinary excretion of markers of collagen breakdown (i.e. pyridinium cross-links) [12, 13] was found to be increased in some AS patients [14, 15].

Since previous studies had suggested a possible pathological role for IGF-I and IGFBP-3 in the aetiology of common OP, and these factors could reflect osteoblast function, we aimed to measure the circulating IGF-I and IGFBP-3 in AS patients.

PATIENTS AND METHODS

Patients

Thirty-three patients satisfying the modified New York criteria for AS [16] were studied. This group...
included 23 males and 10 females. The mean age was 38.5 yr (± 14.8) and the mean disease duration was 6.2 yr (± 5). Twenty-five patients had the HLA B27 antigen. There were no post-menopausal women. No patient had psoriasis or inflammatory bowel disease. All the patients took non-steroidal anti-inflammatory drugs and 14 had received corticosteroids (administration of steroids was intermittent with a daily dose of <15 mg prednisolone and during a maximal period of 3 months). No steroids were given 3 months prior to inclusion in this study. Twenty-two patients had exclusive axial disease and 11 peripheral disease with evidence of arthritis (swollen joint or joint effusion). The radiological sacroiliac joint changes (sacroilitis) and the dorsolumbar X-ray features (for the presence of syndesmophytes) were analysed for the study group. The patients were assessed by two physicians (ET, DW) for Schober’s test. A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index; BASDAI) [17] was also evaluated. Laboratory activity was assessed by the Westergren erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP).

Patients excluded from this study corresponded to post-menopausal women, patients with a condition or a treatment which might alter the bone mineral metabolism (Paget’s disease, hyperthyroidism, hyperparathyroidism, ongoing corticosteroid therapy, thyroxine and anticonvulsants). Patients with diabetes mellitus, obesity, underweight or liver disease were also excluded. Obesity was defined by a body mass index (BMI) between 30 and 40 kg/m² (subjects with a BMI of >40 kg/m² were also considered obese) [18] and subjects with a BMI of <18 kg/m² were defined as underweight.

Controls

The control group included 23 healthy volunteers (mean age 35.8 ± 9.5 yr; 13 males and 10 females; no post-menopausal women) without a history of inflammatory rheumatic disease or condition responsible for bone loss. The exclusion criteria were the same as for the AS group.

Methods

The time of serum sample collection was 8.00 a.m. Fasting venous blood samples were taken from each patient and control, and the serum stored at −20°C. Serum OC concentration, total alkaline phosphatases (tAP), parathyroid hormone (PTH) and 25OHD₃ serum levels were determined using commercial kits [radioimmunoassay (RIA), ost K-PR, Cis-Bio, Gif-sur-Yvette, France; IRMA, Nichols, CA, USA; RRA, Nichols, CA, USA, respectively]. GH, IGF-I and IGFBP-3 concentrations were measured by specific RIA assays (IRMA, Immunotech, France). Insulin serum levels were also evaluated (EIA Tosoh, AIA Pack, France). CRP and ESR were determined by routine laboratory procedures, as well as calcemia and fasting glycaemia.

BMD was measured at the lumbar spine and the femoral neck by dual X-ray absorptiometer (SOPHOS XRA, Sopha Medical, Buc, France). Results were given as bone mineral density and T score, which correspond to the number of standard deviations of any result from the peak bone mass-related population mean (the normal ranges were provided by the manufacturers of the bone densitometer). The BMI was determined for patients and controls.

Statistical analysis

Results are given as the mean ± s.d. Statistical significance was estimated by Student’s t-test. The correlation between variables was analysed by Spearman’s test. The level of significance was <0.05.

RESULTS

Details on the clinical and radiological features of the AS group are summarized in Table I. The levels of the different serum bone markers (calcemia, PTH, 25OHD₃, tAP and OC) were in the normal range without difference between AS and controls (Table II).

As expected, the lumbar spine BMD was significantly lowered in AS patients as compared to controls (P = 0.05) and the corresponding T score was also decreased in this group (P = 0.04). The femoral neck BMD and T score were also found to be decreased in the patient group, but results did not reach the significant level (BMD, P = 0.19; T score, P = 0.17) (Table II). Lumbar spine and femoral neck BMD did not differ between patients who had had corticosteroids and patients who had not lumbar spine BMD: AS with previous steroid administration = 0.96 g/cm² (T score = −1.32) and AS without steroids = 0.94 g/cm² (T score = −1.44) (P = 0.65); femoral neck BDM: AS with previous steroid administration = 0.74 g/cm² (T score = −1.63) and AS without steroids = 0.80 g/cm² (T score = −1.11) (P = 0.22).

The mean values of the BMI were similar in AS and controls.

There were no significant differences in the mean values for the serum parameters including insulin, GH and IGF-I. Although no subjects had diabetes mellitus, AS patients were found to have higher glycaemia than

| TABLE I

| Clinical, biological and radiological characteristics of ankylosing spondylitis patients |
|-----------------|----------|----------|----------|
|                | Mean     | Range    | Median   |
| n               | 33       |          |          |
| Age (yr)        | 38.5     | 22–71    | 29       |
| Sex             | 23 males |          | 14.8     |
|                 | 10 females|          |          |
| Disease duration (yr) | 6.2   | 1–18     | 5        |
| Schober’s test (cm)    | 3.3    | 1–5      | 1.1      |
| Sacroilitis       | 24/33    |          |          |
| Dorsolumbar syndesmophytes | 8/33 |          |          |
| Erythrocyte sedimentation rate (mm/h) | 19.3 | 2–60 | 18 | 16.4 |
| C-reactive protein (mg/l) | 10.0 | 2-51 | 3.5 | 12.4 |
| HLA B27          | 25 positive/33 |          |          |
| BASDAI (0–10)    | 5.0      | 0.4–8    | 5.8      |
|                  |          |          | 2.4      |
controls \( (P = 0.02) \). However, all the glycaemia values were in the normal range \( (3.83–6.05 \text{ mmol/l}) \). Levels of IGFBP-3 were significantly reduced in AS patients \( (P = 0.05) \).

In the patient group, no correlation was observed between the IGF-I or IGFBP-3 serum concentrations and the lumbar spine and femoral neck BMD. Furthermore, there were no relationships between the IGF-I serum values and the different indices of bone turnover \( (\text{PTH}, \text{calcaemia}, 25\text{OHD}_3, \text{tAP}) \) or OC. Indeed, there was a positive correlation between serum levels of IGF-I and OC \( (r = 0.39; P = 0.02) \). No similar result was observed for IGFBP-3 and OC \( (r = 0.02; P = 0.09) \) nor were there any relationships between the serum IGFBP-3 levels and other markers of bone turnover (calcaemia, PTH, tAP and \( 25\text{OHD}_3 \)).

Finally, IGFBP-3 correlated negatively with ESR \( (r = -0.39; P = 0.025) \), but there was no relationship between IGF-I and this parameter of inflammation. Furthermore, no correlation of IGF-I and IGFBP-3 with CRP and the BASDAI could be shown.

**DISCUSSION**

Our study was undertaken to determine the serum levels of IGF-I and IGFBP-3 as markers of bone formation in AS. There is growing evidence that this bone growth factor and its main binding protein are clearly important factors in bone physiology and pathology \([6, 7, 19]\). Indeed, IGF-I has a marked anabolic effect on bone by different mechanisms and is implicated in OP at different levels \([6]\). A negative relationship between age and plasma IGF-I levels was found, as well as bone density decreasing with age \([19]\). A parallel decline in serum and skeletal IGF-I in aging human was reported \([19]\). It has been demonstrated that IGF-I was directly related to axial bone density in post-menopausal women \([7]\), that IGF-I serum levels were decreased in post-menopausal OP \([8]\) and that male patients with idiopathic OP also had a lowered concentration of this growth factor \([9]\). The importance of IGF-I in the mechanism of OP was highlighted by treatment of male idiopathic OP patients with IGF-I as anabolic therapy \([20]\). This treatment enhanced bone formation, as was demonstrated by increased serum levels of OC and procollagen peptides. However, a parallel increase in urinary excretion of collagen cross-links indicated stimulation of bone remodelling in these patients. In addition, serum IGF-I could be considered as a marker of bone formation; it was evaluated in patients with idiopathic OP and compared to histological findings. A good correlation between serum IGF-I and histomorphometric indices of bone formation (osteoblastic surface and mineralizing bone surface) was observed \([21]\).

IGFBP-3 is the predominant IGFBP for IGF-I that is synthesized by the osteoblast cell lineage \([6, 19]\). The role of this protein, as well as the others, is still not well understood, but it is thought that it modulates growth factor availability and activity. Serum levels of IGFBP-3 are dependent on GH and thus reflect GH secretion. GH stimulates accumulation of IGFBP-3 by osteoblasts and IGFBP-3 may increase the biological action of IGF-I in bone \([6]\). There were some implications of this IGFBP in OP since serum IGFBP-3 was found to be decreased in post-menopausal OP \([8]\). All these recent data suggested that alteration in the synthesis of IGF-I, its receptor binding or IGFBP could play a role in the pathogenesis of OP \([19]\).

In AS, bone loss leading to generalized OP is a complicating feature of the disease \([1]\). We have measured BMD in AS patients and found a significantly lowered BMD and T score at the lumbar spine. These data are consistent with previous studies of BMD assessment in AS \([4]\). In this series of patients with lowered spine BMD, and on the contrary in post-menopausal OP, we found no decreased concentrations of IGF-I. However, serum levels of IGFBP-3 were significantly lower amongst patients than controls. It has been demonstrated that IGFBP-3 plays an important role in the IGF-I activity in osteoblastic cells. Therefore, it may be hypothesized that the decreased concentrations of IGFBP-3 could impair the activity of this growth factor in our patients.

Another implication of this data is the regulation of IGF-I and IGFBP-3 by GH. Both are dependent on GH itself and reflect endogenous GH secretion. In our study, no differences in the basal serum values of GH were observed between patients and controls. Since the determination of IGF-I levels was normal in AS, it is difficult to speculate diminished GH secretion. However, no stimulating tests for GH secretion were performed.
IGF-I and its IGFBPs have not been previously evaluated in adult patients with chronic inflammatory diseases. They were studied in juvenile chronic arthritis (JCA) because these conditions are frequently complicated by growth delay [22]. Several studies had therefore focused on the determination of IGF-I levels in JCA patients. Results were conflicting, but most studies found decreased IGF-I serum values in JCA. A negative correlation between serum IGF-I and ESR was recently reported, and it was hypothesized that inflammation might play a role in decreasing serum levels of IGF-I [22]. Inflammation could interfere, by means of different cytokines (interleukin-1, interleukin-6 or tumour necrosis alpha), with IGF-I production in response to GH stimulus. According to these data, the same explanation could be suggested for the reduced serum levels of IGFBP-3 and its relationship (negative correlation) with ESR in our study. Thus, inflammation could be a factor that impairs the synthesis of IGFBP-3 in response to GH. However, the normal value of the serum concentration of IGF-I in our patients remains unclear. Moreover, these findings are not specific for an inflammatory rheumatic disease since similar involvement of IGF-I/IGFBP was obtained in AS and JCA.

It is well known that relevant variables of circulating IGF-I include age and body mass [6]. However, in our study, subjects with obesity or underweight were excluded and the mean age of controls and AS patients was 35.8 and 38.5 yr, respectively. Furthermore, no subjects had liver disease that could interfere in the determination of IGF-I.

Since our study found decreased IGFBP-3 serum levels and this IGFBP is an important cofactor for IGF-I, it could be speculated that this reflects impairment in osteoblast function by a decrease in local concentrations of IGF-I, and thus could affect bone formation. In AS, biochemical markers of bone formation, including OC and alkaline phosphatases, were found to be normal in previous studies [10, 11]. Abnormalities in the assessment of markers of bone turnover in AS include enhanced urinary excretion of pyridinium cross-links [14, 15], a marker of bone degradation [12, 13, 23]. Our data suggested that low bone mass in AS could also be related to bone formation impairment by involvement of the IGFBP-3/IGF-I axis. The results described here may be paralleled by those of a previous study with mineralization defects in histological assessment of AS patients [24].

We conclude that AS patients with decreased lumbar spine BMD had lower IGFBP-3 concentrations and that inflammation might play a role in decreasing the serum levels of this IGFBP. It is likely that these changes contribute to impaired osteoblastic function by a decrease in local bone concentration of IGF-I. However, assessment of other bone growth factors (IGF-II) and IGFBPs (IGFBP-1 and -4) is required. Thus, the role of each of these bone growth factors and their IGFBPs in the pathogenesis of AS OP remains to be clarified.

Acknowledgements

This work was supported by a grant from La Societé Française de Rhumatologie.

References

5. Will R, Bhalla AK, Palmer R, Ring F, Calin A. Decreased serum levels of insulin-like growth factors was obtained in AS and JCA.
7. Marie P. Facteurs de croissance et formation ossee dans les oste´ope´ nies: roˆles de l'IGF-I et du TGF

8. Risteli L, Risteli J. Biochemical markers of bone metab-

9. Ljunghall S, Johansson AG, Burman P, Kämpe O, Lindh E, Karlsson FA. Low plasma levels of insulin-like growth factor 1 (IGF-1) in male patients with idiopathic osteopo-

13. Risteli L, Risteli J. Biochemical markers of bone metab-

14. Marho

15. Macdonald AG, Birkinshaw G, Durham B, Bucknall RC, Fraser WD. Biochemical markers of bone turnover in seronegative spondylarthropathy: relationship to dis-

16. Van Der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposa-