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THE SERUM GROWTH HORMONE TO SOMATOSTATIN RATIO IS SKEWED UPWARD IN RHEUMATOID ARTHRITIS PATIENTS

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1. ABSTRACT

Basal serum growth hormone, insulin-like growth factor-1 (IGF-1) and somatostatin concentration were measured by standard radioimmunoassay in patients with a diagnosis of rheumatoid arthritis (RA) according to the criteria of the American College of Rheumatology as well as in a group of age-matched normal subjects. RA patients exhibited significantly elevated (age, 45-55 yrs, p less than 0.05; 55 yrs and older, p less than 0.01) serum growth hormone levels compared to age-matched individuals from the control group. IGF-1 was unchanged. Serum somatostatin levels were reduced in RA patients between 45 and 55 yrs but reached a significant reduction (p less than 0.0001) in RA patients, 55 years and older compared to age-matched individuals from the control group. RA patients treated with prednisone did not exhibit changes in either growth hormone or IGF-1 levels compared to RA patients treated principally with non-steroidal anti-inflammatory drugs and methotrexate. These results indicated that symptomatic RA is associated with elevated serum growth hormone without concomitant changes in IGF-1 compared to individuals from the control group. Reduced somatostatin levels in older RA patients resulted in a skewed upward growth hormone to somatostatin ratio. We conclude that the serum growth hormone to somatostatin ratio may be a useful surrogate marker of disease activity in symptomatic RA.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder of unknown etiology characterized by altered cellular and humoral immunity (1). Symptomatic RA patients present with joint inflammation characterized by concomitantly elevated arachidonic acid metabolites, vasoactive amines, neuropeptides and cytokines that appear to be the basis for joint pain and other systemic manifestations of RA. We previously showed that patients with rheumatic disorders, notably, osteoarthritis (OA) (2-5), diffuse idiopathic skeletal hyperostosis (DISH) (5, 6) as well as hypermobility syndrome (7) had elevated serum (2-7) and synovial fluid growth hormone concentrations (3). In most rheumatic disorders, higher serum growth hormone levels resulted in elevated insulin-like growth factor (IGF-1) levels (3, 5, 6, 7), but not in OA, where IGF-1 levels were reduced compared to normal (2, 3, 5). Of note, conventional medical therapy including non-steroidal anti-inflammatory drugs (NSAIDs) reduced OA and DISH clinical symptoms, including pain, which was associated with decreasing serum growth hormone concentrations approaching levels measured in normal subjects (6, 8). Further, we recently demonstrated that erythrocytes derived from OA and DISH patients sequestered growth hormone in amounts that exceeded serum levels (9). This suggested a mechanism whereby transport to, and release of "toxic" growth hormone levels could occur in synovial joints (4) with potential exacerbation of inflammatory arthritis.

Somatostatin is a 14 amino acid polypeptide widely distributed throughout the central nervous system and peripheral tissues which was originally identified on the basis of its ability to inhibit growth hormone release from the pituitary (10). In the context of RA, several studies have suggested a role for somatostatin in arthritis animal models. Thus, in chronic rat adjuvant-arthritis, immunohistochemical labeling and electron microscopy identified somatostatin in mature bone matrix, monocytes, bone marrow neutrophils and macrophage-like synoviocytes (11). In a fibrin-induced lapine arthritis model, somatostatin caused a significant and dose-related attenuation of knee joint swelling (12).

Table 1. Serum Growth Hormone and Somatostatin Concentration in Normal Subjects and Rheumatoid Arthritis Patients

Measurement	Age Group	45-55 yrs	55 yrs and older
Growth Hormone [1]	Normal	1.06 ± 0.37	1.09 ± 0.83
	RA	1.72 ± 1.33	1.85 ± 1.42
p-value		Less than 0.05	Less than 0.01
Somatostatin [2]	Normal	32.6 ± 12.0	36.2 ± 10.0
	RA	29.5 ± 11.0	25.0 ± 9.0
p-value		Greater than 0.05	Less than 0.0001

[1] ng/ml (Mean ± SD), [2] pg/ml (Mean ± SD)

In addition to animal studies, somatostatin was found to inhibit human RA synovial cell proliferation as well as cytokine and matrix metalloproteinase levels (13, 14). Further, somatostatin up-regulated the apoptosis modulators, p53, Bcl-2, Fas and caspase-8 while down-regulating inducible nitric oxide synthase and nitric oxide in thioglycolate-elicited peritoneal macrophages (15) suggesting that persistent aberrant proliferation and apoptosis-resistance typical of RA synovium (16) could potentially be modulated by administration of exogenous somatostatin.

In a preliminary study of 24 RA patients, we found that serum growth hormone levels were significantly elevated in male RA patients compared to a group of normal male subjects and that synovial fluid growth hormone levels were increased 3-fold compared to serum growth hormone levels in female RA patients (3). The present investigation was designed to extend those studies and establish the extent to which serum growth hormone, IGF-1 and somatostatin were altered in a larger group of symptomatic RA patients as well as to assess the effect of prednisone therapy on serum growth hormone and IGF-1 levels. The results established that serum growth hormone was elevated in RA patients compared to individuals in a normal age-matched control group, whereas IGF-1 was unchanged. However, prednisone failed to change either serum growth hormone or IGF-1. Further, serum somatostatin was reduced, reaching significance in RA patients 55 yrs and older. This resulted in a skewed upward growth hormone to somatostatin ratio.

3. MATERIALS AND METHODS

3.1. Patient Population

This study was performed at University Hospitals of Cleveland (UHC) and the Wade Park Veterans Administration Medical Center (VAMC) (Cleveland, Ohio). The UHC and VAMC Institutional Review Boards approved the study design and protocol which required informed consent to be obtained according to the Declaration of Helsinki. Participants were all volunteers, 21 yrs and older. For inclusion in the RA group, patients had to meet criteria for the diagnosis of RA established by the American College of Rheumatology (17).

Fifty-six RA patients and 28 normal subjects were studied. Females comprised 87.5% of the RA study group of which 47.6% were African-American (58.4±11.3 yrs, mean ± SD) and 42.4% were Caucasian (63.8 ± 10.7 yrs). A control group was age-matched to the RA group. Serum growth hormone and IGF-1 levels were also studied in 24 female RA patients treated with prednisone (35-70mg/week) and 25 female RA patients receiving other medical therapies, which included primarily, NSAIDs, methotrexate (5-10mg/week) or sulfasalizine, but excluding corticosteroids at the time blood was obtained.

3.2. Blood Sample Collection and Determination of Blood Glucose

Blood drawn by venipuncture was clotted at room temperature, centrifuged, serum aliquots separated, and serum stored at -70°C. Blood samples were obtained during a 3-4 hr morning period in order to limit the contribution of growth hormone pulses and serum glucose levels to serum growth hormone measurements (6). Growth hormone, IGF-1 and somatostatin assays were performed only if serum glucose was in the euglycemic range (i.e. 65-130 mg/dl) attained by overnight fasting or a fast of at least 4 hrs or more.

3.3. Serum growth hormone, IGF-1 and somatostatin radioimmunoassay

Standard radioimmunoassay (RIA) (INCSTAR, Stillwater, MN) was adapted for growth hormone, IGF-1 and somatostatin. The manufacturer provided quality control samples for each peptide with each kit. RIA including standard curves and intra-kit variability tests were performed as previously described (2-9).

3.4. Data Analysis

The 2-tailed t-test was employed to analyze the significance of means between groups of unequal size where p less than 0.05 indicated significance (2-9). Serum IGF-1 (2-5) and somatostatin minimal detectable levels as indicated by the manufacturer were exceeded in all serum samples. However, the minimal detectable serum growth hormone_concentration was 0.4ng/ml (2-9). Serum growth hormone values less than 0.45ng/ml (5.4% of the samples) were excluded from the statistical analysis and for technical reasons the serum IGF-1 concentration in one female RA patient not treated with prednisone (see below) was not determined.

4. RESULTS

4.1. Growth hormone and somatostatin

Basal serum growth hormone was significantly increased in RA patients compared to individuals in the control group of normal subjects (Table 1). By contrast, basal serum somatostatin levels were lower in RA patients in the 45-55 yrs group compared to individuals in the control group reaching significance in RA patients, 55 yrs and older (Table 1). The growth hormone to somatostatin ratio was relatively constant in individuals from the control groups (i.e. 45-55 yrs, 32.5; 55 yrs and older, 30.1) but skewed upward in the RA groups (i.e. 45-55 yrs, 58.3; 55 yrs and older, 74.0).

Table 2. Serum Insulin-like Growth Factor-1 (IGF-1) Concentration in Normal Subjects and Rheumatoid Arthritis Patients

	45 – 55 yrs		55 yrs and older		All Patients	
Group	Normal	16.6±3.7[1]	Normal	19.7±7.1	Normal (28)	22.9±7.9
Group	RA	22.4±9.0	RA	17.8±7.3	RA (49)	19.7±8.3
	p-value	Greater than 0.05		Greater than 0.05		Greater than 0.05

[1] nmol/L (Mean ± SD)

Table 3. Prednisone Does Not Alter Serum Growth Hormone (GH) or Serum Insulin-like Growth Factor-1 (IGF-1) Levels in Rheumatoid Arthritis Patients

GH (–Prednisone)	GH (+Prednisone)	IGF-1 (-Prednisone)	IGF-1 (+Prednisone)
2.17 ± 1.67 [1]	1.45 ± 1.04	17.2 ± 8.3 nmol/L	21.7 ± 8.1
N=25	N=24	N=24	N=24
p-value	Greater than 0.05		Greater than 0.05

[1] ng/ml (Mean ± SD)

4.2. Growth Hormone and IGF-1

Serum IGF-1 levels did not differ among the groups (Table 2). Serum growth hormone and IGF-1 levels in female African-American RA patients were 1.66 ± 0.87 ng/ml and 21.4 ± 8 nmol/L, respectively, compared to 2.12 ± 1.88 ng/ml and 17.2 ± 8.5 nmol/L in the female Caucasian RA group.

4.3. Effect of prednisone on growth hormone and IGF-1

Although prednisone therapy reduced basal serum growth hormone levels, this did not reach significance compared to the non-prednisone-treated RA group (Table 3). Further, prednisone did not alter IGF-1 levels (Table 3).

5. DISCUSSION

This study demonstrated that basal serum growth hormone levels were elevated while somatostatin levels were reduced in symptomatic RA resulting in a skewed upward growth hormone to somatostatin ratio. The growth hormone to somatostatin ratio in normal subjects remained in a constant range independent of age, whereas in the RA patient groups, the growth hormone to somatostatin ratio was elevated in the 45-55 yrs RA group and was further increased in the 55 yrs and older RA group (Table 1). The higher growth hormone to somatostatin ratio in the older RA patients may be associated with RA of longer duration.

Serum growth hormone levels were higher in female Caucasian RA patients than in female African-American RA patients. We had previously also shown this to be the case in female Caucasian and African-American normal subjects where the values for growth hormone were 1.1 ± 0.2ng/ml and 0.8 ± 0.5ng/ml (mean ± SD), respectively (3). Further, the RA serum growth hormone levels measured in the present study were greatly elevated (?%, 92.7, female Causcasian; ?%, 107.5, female African-American) compared to individuals in the control group subdivided by race.

Although there was a trend towards reduced serum growth hormone levels in those RA patients treated with prednisone, the reduction in growth hormone when prednisone-treated and non-prednisone-treated RA patients were compared did not reach significance. Thus, the present results differ from previous studies where OA and DISH clinical improvement by means of conventional use of NSAIDs and other anti-inflammatory drugs resulted in a significant reduction in growth hormone levels to those levels found in normal subjects (6, 8). We have not ascertained the effect of corticosteroids on basal serum somatostatin in RA patients, although a previous study suggested that the low doses of corticosteroids commonly employed in RA therapy were unlikely to affect growth hormone release (18).

In the present study, serum IGF-1 levels in RA patients did not differ from individuals in the control group. Previous studies have shown that IGF-1 levels may be regulated by nutritional intake independent of growth hormone (19). Although serum growth hormone is elevated in many rheumatic disorders studied thus far (2-9), IGF-1 levels are lower than expected in OA, if growth hormone is coupled to IGF-1 (2, 3, 5). Countervaling evidence has been presented which showed no reduction in IGF-1 in knee OA compared to normals after adjusting for age-related changes in IGF-1 (20). However, we previously showed that lowering basal serum growth hormone (as a function of clinical improvement in OA) to levels approaching those found in a normal control group also resulted in increased IGF-1 levels (8). This suggested that in achieving clinical improvement in OA (i.e. asymptomatic OA), serum growth hormone changes were coupled to serum IGF-1 changes. By contrast, in patients with DISH, clinical improvement resulted in lowered basal serum growth hormone without a concomitant change in IGF-1 (6). In the present study of RA, prednisone altered neither growth hormone nor IGF-1 levels suggesting that persistently high levels of growth hormone are likely to play a role in RA progression despite the strong radiographic evidence that oral corticosteroids affect a reduction in the progression of cartilage erosions (21,22).

A previous study showed impaired hypothalamic hormone release and growth hormone secretion in active and remitted RA patients after insulin-induced hypoglycemia (23). The present study included normal subjects and RA patients only if their fasting serum glucose was in the euglycemic range. Differences in study design make it is difficult to directly compare the extent to which serum glucose levels contributed to the lower growth

hormone levels reported in the previously published study (23).

Serum somatostatin levels measured in RA patients (Table 1) were similar to those levels previously reported by Marabini *et al.* (24) in RA synovial fluid. They demonstrated that somatostatin levels were higher in RA synovial fluid compared to OA and psoriatic arthritis synovial fluid. However, a group of normal subjects was not included for comparison in that study (24). In support of the present findings, Matucci-Cerinic *et al.* (12) suggested that significantly lower somatostatin levels in RA may also contribute to synovial joint inflammation as was seen in experimental arthritis. Further, Vanhagen *et al.* (25) showed that RA clinical activity measured principally as the number of swollen and painful joints correlated with somatostatin receptor levels measured by scintigraphy. Somatostatin receptors were also localized to RA synovial membrane (25).

It is reasonable to conclude based on accumulated evidence that serum and synovial fluid growth hormone and somatostatin, acting in concert with other acute phase reactants contributes, in part, to the inflammatory component of adult rheumatic diseases. Thus, growth hormone and somatostatin should be added to a growing list of markers of RA disease activity which include, hyaluronate, aggrecan, Type II collagen fragments, cartilage oligomeric matrix protein (COMP), extraarticular cartilage matrix protein (CMP) as well as the more conventional non-specfic markers of inflammation exemplified by erythrocyte sedimentation rate and C-reactive protein (26).

Although it is presently unknown the extent to which intra-articular somatostatin administration might be a useful adjunctive RA therapy or even a substitute for corticosteroids, methotrexate, NSAIDs or tumor necrosis factor monoclonal antibody inhibitors, the results of the present study suggested that lowering the growth hormone to somatostatin ratio to that measured in normal subjects may be desirable to suppress joint inflammation while also potentially inducing apoptosis in the hyperactive proliferative RA synovium. In this respect, intra-articular somatostatin adminstration to 10 RA patients produced a transient benefit by partially resolving RA symptoms (27).

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