Gelsolin levels are decreased in ankylosing spondylitis patients undergoing anti-TNF-alpha therapy

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Abstract

Objective

To determine whether circulating gelsolin (GSN) levels in patients with ankylosing spondylitis (AS) undergoing TNF-α antagonist-infliximab-therapy are altered compared with controls and to establish whether disease activity, systemic inflammation and metabolic syndrome are potential determinants of circulating GSN levels in these patients.

Methods

We assessed GSN serum concentrations in a series of 30 non-diabetic AS patients without cardiovascular (CV) disease undergoing TNF-α antagonist-infliximab therapy and 48 matched controls. GSN levels were measured immediately before and after an infliximab infusion. Correlations of GSN serum levels with disease activity, systemic inflammation and metabolic syndrome were assessed. Potential changes in GSN concentration following an infusion of anti-TNF-α monoclonal antibody-infliximab were also analysed.

Results

Although at the time of the study AS patients undergoing anti-TNF-α therapy had adequate control of the disease (mean BASDAI 2.94), they showed lower GSN serum levels than healthy controls (mean±SD: 38660.42±23624.6 ng/ml versus 68975.43±31246.79 ng/ml; p<0.0001). When AS patients were stratified according to sex, we observed that GSN levels were significantly lower in men than in women (p=0.032). However, no differences in GSN levels according to the specific clinical features of the disease were seen. No association was found between GSN concentration and adipokines or biomarkers of endothelial cell activation. However, correlation between basal GSN levels and insulin resistance was observed. A single infliximab infusion did not lead to significant changes in GSN levels.

Conclusions

GSN concentration is reduced in AS patients undergoing periodical anti-TNF-α therapy and low disease activity. Potential association with some metabolic syndrome features seems to exist.

Key words

ankylosing spondylitis, atherosclerosis, inflammation, anti-TNF- antibody-infliximab, gelsolin

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease characterised by predominant axial joint involvement, sacroiliitis and some extra-articular manifestations. Patients with AS have 1.5–2 times higher cardiovascular (CV) mortality than the normal population (1, 2), and this is probably due to a process of accelerated atherosclerosis (3). Endothelial dysfunction and increased common carotid artery intima-media wall thickness have been reported in AS patients (4-6). These findings indicate the presence of subclinical atherosclerosis associated to the disease (7).

Traditional CV risk factors such as obesity and its related metabolic syndrome contribute to the increased CV morbidity and mortality observed in AS patients (8). The chronic pro-inflammatory state present in AS patients is an additional CV risk factor (2). In this context, gelsolin (GSN), a protein involved in cytoskeleton reorganisation, could play a key role. GSN is a protein secreted by many cell types, being mainly synthesised by the muscle cells (9). This protein has two different isoforms: a cytoplasmic and a circulating isoform (10). Gelsolin (GSN) induces the depolymerisation of actin filaments, preventing thus inflammatory reactions which would otherwise be stimulated downstream by these actin filaments (11). In situations of acute injury or inflammation, GSN levels tend to decrease (10-12). In this regard, in inflammatory diseases such as RA, circulating GSN may be potentially locally consumed by the interaction with different macromolecules (actin, fibrin and fibronectin) at the joints or other affected organs (11). By binding to these macromolecules at these sites, circulating GSN levels tend to decrease (11). Based on this fact and on the observation that GSN null mice respond more slowly to an induced inflammatory stimulus (13), it was suggested that GSN could be a potential molecule for anti-inflammatory treatment. Besides, exogenous administration of recombinant GSN in mice diminishes inflammatory responses (14). An additional evidence of the pivotal role of GSN was reported by Lee *et al*., who observed lower levels of GSN in patients undergoing haemodialysis, when compared to healthy controls. They also demonstrated a correlation between low GSN levels and a higher risk of haemodialysis mortality (15).

To determine the possible link between chronic inflammation and metabolic syndrome with atherosclerosis in AS, we previously analysed the effect of the treatment with the anti-TNF- monoclonal antibody-infliximab in a series of non-diabetic AS patients undergoing periodic treatment with this drug. We observed that infliximab treatment reduced significantly serum insulin levels and improved insulin sensitivity (16). To further establish potential beneficial effects mediated by the anti-TNF-α blockade on the metabolic syndrome associated to AS, we also studied serum levels of several adipokines in non-diabetic AS patients undergoing infliximab therapy. We found that adiponectin serum levels positively correlated with insulin sensitivity, suggesting that low circulating adiponectin concentrations may be involved in the pathogenesis of the CV disease in AS (17). In assessing visfatin serum levels in the same population, we also disclosed a significant positive correlation of this adipokine with insulin resistance (18). Because of that, we also analysed apelin serum levels, a new adipokine recently involved in CV risk, but we could not find apelin association with disease activity or with metabolic syndrome (19).

Treatment with anti-TNF-α agents has been found to be effective in patients with AS and other spondyloarthropathies (20-22). As discussed before, a rapid beneficial improvement of insulin sensitivity mediated by infliximab was also observed (16). Therefore, it is plausible to think that TNF-α blockade might account for biological changes that may slow the progression of atherosclerosis in patients with AS. Hence, the establishment of potential changes in adipokines and biomarkers of endothelial cell activation, endothelial dysfunction and inflammation following the administration of anti-TNF-α drugs in AS patients may improve our understanding of the effect of these biologic agents in this pathology.

Taking these considerations together, in the present study we aimed to establish whether in non-diabetic AS patients undergoing periodic treatment with infliximab GSN levels are different from those of controls. Also, we aimed to establish whether inflammation and/or metabolic syndrome have any influence on circulating GSN concentrations and whether possible associations of circulating GSN concentrations with clinical and demographic characteristics may exist in these patients. Moreover, we investigated the effect of an infliximab infusion on circulating GSN concentrations in that series of non-diabetic AS patients who required this therapy because of disease refractory to non-steroidal anti-inflammatory drugs (NSAIDs).

Patients and methods

Patients

We assessed a series of 30 patients with AS attending hospital outpatient clinics seen over 14 months (January 2009 to March 2010), who fulfilled the modified New York diagnostic criteria for AS (23). They were treated by the same group of rheumatologists and were recruited from the Hospital Xeral-Calde, Lugo, Spain. For the comparative analysis with AS patients we used 48 controls matched by age, sex, ethnicity and traditional CV risk factors, who did not have history of CV events.

For ethical reasons, patients included in the present study were not randomised to a placebo group. The same procedure has been found acceptable and followed in studies on the short term effect of infliximab therapy on the lipid profile, adipokines and biomarkers of endothelial cell activation in patients with rheumatoid arthritis (RA) (24-26).

Patients on treatment with infliximab seen during the period of recruitment with diabetes mellitus or with plasma glucose levels greater than 110 mg/dl were excluded. None of the patients included in the study had hyperthyroidism or renal insufficiency. Also, patients seen during the recruitment period who had experienced CV events, including ischaemic heart disease, heart failure, cerebrovascular accidents or peripheral arterial disease were excluded. Hypertension was diagnosed in patients with a blood pressure of ≥140/90 mmHg and in those taking antihypertensive agents. Obesity was defined if body mass index (BMI) (calculated as weight in kilograms divided by height in squared meters) was greater than 30.

In all cases the anti-TNF-α monoclonal antibody-infliximab was prescribed because of active disease. All patients included in the current study had begun treatment with NSAIDs immediately after the disease diagnosis. All of them were still being treated with these drugs at the time of the study. At the time of this study most patients were on treatment with naproxen: 500-1000 mg/d. Although the 2010 updated recommendations facilitate initiation of TNF-α blockers in AS and only ask for 2 NSAIDs with a minimum total treatment period of 4 weeks (27), for the initiation of anti-TNF-α therapy in these series of patients recruited between January 2009 to March 2010, they had to be treated with at least 3 NSAIDs prior to the onset of infliximab.

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI- range of 0 to 10) (28) was evaluated in all patients at the time of the study. Clinical information on hip involvement, history of synovitis in other peripheral joints and peripheral enthesitis, history of anterior uveitis, presence of syndesmophytes and HLA-B27 status (typed by cell cytotoxicity) was assessed. Moreover, C-reactive protein (CRP)- by a latex immunoturbidity method, erythrocyte

Table I. Demographic, clinical and laboratory data of 30 patients with ankylosing spondylitis.

Variable	n (%)
Mean age (years) ± SD	
At the time of study	50.47 ± 14.85
At the time of onset of symptoms	28.23 ± 10.40
Delay to the diagnosis (years) ± SD	11.48 ± 9.01
Men/Women	21 (70) / 9 (30)
Mean disease duration (years) ± SD*	21.97 ± 13.16
History of classic cardiovascular risk factors	
Hypertension	12 (40)
Dyslipidaemia	11 (36.67)
Obesity (BMI >30 kg/m^2)	3 (10.00)
Current smokers	13 (43.33)
Mean blood pressure (mm Hg) ± SD*	
Systolic	123.17 ± 18.17
Diastolic	75.67 ± 12.51
Mean body mass index (kg/m2) ± SD	26.70 ± 3.26
Mean BASDAI ± SD*	2.94 ± 2.11
Mean VAS spinal pain ± SD*	31.13 ± 24.23
Hip involvement	6 (20)
Synovitis in other peripheral joints and peripheral enthesitis	11 (36.67)
Anterior uveitis	6 (20.00)
Syndesmophytes	10 (33.33)
Mean CRP (mg/l) ± SD**	
At the time of disease diagnosis	24.01 ± 33.43
At the time of study	6.24 ± 8.65
Mean ESR (mm/1st hour) ± SD***	
At the time of disease diagnosis	30.10 ± 28.23
At the time of study	19.00 ± 15.18
Mean cholesterol or triglycerides (mg/dl) ± SD*	
Total cholesterol	199.10 ± 30.61
HDL cholesterol	53.17 ± 12.81
LDL cholesterol	126.77 ± 26.54
Triglycerides	93.97 ± 56.70
Mean fasting serum glucose (mg/dl) ± SD*	92.77 ± 8.63
HLA-B27 positive (n=27)	20 (74.07)

*At the time of the study. **Normal value <5 mg/l. ***Normal value < 20 mm/1st hour.
BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HLA: human leukocyte antigen; LDL: low-density lipoprotein; SD: standard deviation; VAS: visual analogue scale

sedimentation rate (ESR)-Westergren, serum glucose, total cholesterol, HDL and LDL cholesterol and triglycerides (fasting overnight determinations) were assessed in all the patients at the time of the study.

The main demographic, clinical and laboratory data of this series of 30 AS patients at the time of the study are shown in Table I. Since at that time all patients were undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab (median duration of periodical treatment with biologic agent: 23 months), the mean BASDAI ± standard deviation (SD) was only 2.94±2.11.

The local institutional committee approved anti-TNF-α therapy. Also, patients gave informed consent to participate in this study. Neither this study nor the former studies on the short term effect of infliximab therapy on insulin resistance in AS (16) or adipokines (17, 18) were supported by any pharmaceutical drug company.

Study protocol

In all cases, the drug was given to patients as an intravenous infusion in a saline solution over 120 minutes. All measurements were made in the fasting state. Blood samples were taken at 0800 hours for determination of the ESR (Westergren), CRP (latex immuno-turbidimetry), lipids (enzymatic colorimetry), plasma glucose and serum insulin (DPC, Dipesa, Los Angeles, CA, USA). As previously described, insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) using the formula= (insulin (μU/ml) x glucose (mmol/l)÷22.57 (16). A commercial ELISA kit was used to measure serum GSN levels (Uscn, E90372Hu; assay sensitivity=2.56 ng/ml; intra- and interassay coefficients of variation were <10% and <12%, respectively) (Uscn, Life Science Inc, Houston, TX, USA) according to the manufacturer's instructions. Serum levels of GSN were measured in samples obtained immediately before an infliximab infusion and 120 minutes later (immediately after the infusion). Total plasma adiponectin, serum resistin, leptin, visfatin, apelin, angiopoietin-2 (Angpt-2), asymmetric dimethylarginine (ADMA), and ghrelin levels immediately prior to and after an infliximab infusion were determined by ELISA as previously described (17, 18, 19, 29, 30, 31).

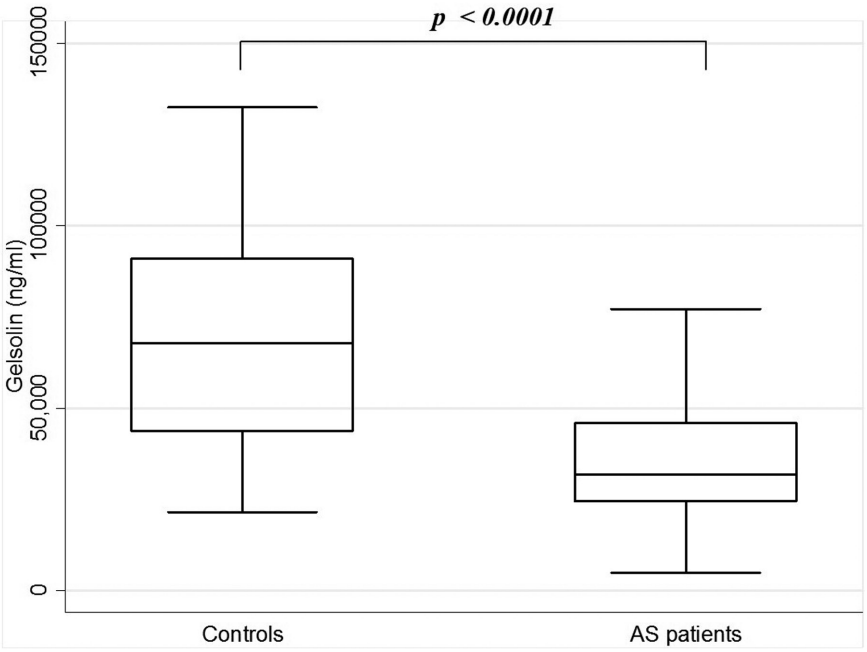

Fig. 1. Box plot showing differences in GSN serum levels between patients with AS and healthy controls.

Statistical analyses

Variables were expressed as mean ± (standard deviation) SD, median (interquartile [IQ] range) or percentages. Correlation between basal GSN at time 0 with selected continuous variables was performed adjusting by age at the time of the study, sex, and classic cardiovascular risk factors via estimation of the Pearson partial correlation coefficient (r).

The associations between baseline characteristics and serum GSN concentrations were assessed by the Student's paired *t*-test for categorical variables. Differences in GSN levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U-test.

GSN serum levels before (time 0) and postinfusion (time 120) were compared using the paired Student *t*-test.

Two-sided *p*-values ≤0.05 were considered to indicate statistical significance. Analyses were performed using Stata 12/SE (StataCorp, College Station, TX).

Results

Differences in gelsolin serum levels between AS patients and controls

GSN serum concentrations were lower in AS patients (mean±SD: 38660.42±23624.6 ng/ml; median [IQ range]: 31830.5 [24560.85–45769.01]) than in healthy controls (mean±SD: 68975.43±31246.79 ng/ml; median [IQ range]: 67697.78 [43558.5–90863]) (p<0.0001) (Fig. 1).

Relationships of demographic features, inflammation, adiposity and adipokines with circulating gelsolin concentration

GSN serum levels did not show significant correlation with age at the onset of symptoms, BMI, CRP and ESR at the time of the study or at the time of disease diagnosis (Table II). Neither did we find any association with resistin, adiponectin, leptin, visfatin, Angpt-2, apelin, ADMA or ghrelin serum levels (Table II). Nevertheless, when we compared GSN serum levels between men and women, we found significantly lower levels of this protein in men (Table III).

Relationships of gelsolin concentration with metabolic syndrome features other than adiposity

We did not observe any statistically significant correlation between GSN

Table II. Partial correlation of serum Gelsolin prior to infliximab infusion (at time 0) with selected continuous variables adjusting by age at the time of the study, sex, and classic cardiovascular risk factors in 30 patients with ankylosing spondylitis.

Variable	Gelsolin	
	r	*p*
Age at the onset of symptoms	0.076	0.70
Disease duration*	-0.098	0.63
BMI*	-0.173	0.39
Systolic blood pressure*	0.083	0.68
Diastolic blood pressure*	-0.028	0.89
BASDAI*	0.316	0.11
VAS spinal pain*	0.256	0.20
ESR* (natural-log-transformed)	0.012	0.95
CRP* (natural-log-transformed)	-0.119	0.55
ESR** (natural-log-transformed)	0.153	0.45
CRP** (natural-log-transformed)	0.146	0.47
Total cholesterol* (natural-log-transformed)	-0.352	0.07
HDL cholesterol* (natural-log-transformed)	-0.336	0.09
LDL cholesterol* (natural-log-transformed)	-0.173	0.39
Triglycerides* (natural-log-transformed)	-0.124	0.54
Serum glucose* (natural-log-transformed)	0.056	0.78
HOMA-IR at time 0*	0.397	0.04
QUICKI at time 0*	-0.467	0.01
Resistin at time 0	-0.224	0.32
Adiponectin at time 0	-0.135	0.51
Leptin at time 0	0.054	0.79
Visfatin at time 0	0.353	0.07
Angpt-2 at time 0	0.129	0.52
Apelin at time 0	0.068	0.74
ADMA at time 0	0.156	0.45
Ghrelin at time 0	-0.257	0.20

*At the time of the study. **At the time of disease diagnosis.
Angpt-2: angiopoietin-2; ADMA: Asymmetric dymethilarginine; BASDAI: Bath ankylosing spondylitis disease activity index; BMI: Body mass index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL: low-density lipoprotein; QUICKI: quantitative insulin sensitivity check index; VAS: visual analogue scale.

serum levels with systolic or diastolic blood pressure, total cholesterol, HDL- and LDL-cholesterol, triglycerides or serum glucose levels (Table II). Besides, no significant differences in GSN concentration were seen when patients were stratified according to the presence or absence of arterial hypertension, dyslipidaemia or obesity (Table III). However, we observed a significant correlation between GSN concentration and insulin resistance (HOMA-IR at time 0) (r=0.397; *p*=0.04) (Table II). We also observed a correlation with insulin sensitivity (QUICKI) (r=-0.467; *p*=0.01) (Table II).

Relationships of gelsolin concentration with other recorded baseline characteristics

Circulating GSN concentration did not correlate with disease duration, BASDAI or VAS spinal pain at the time of the study (Table II). Likewise, no difference in GSN concentration was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis in other peripheral joints and peripheral enthesitis was compared with the remaining patients who did not exhibit these features (Table III). It was also the case when patients were compared according to HLA-B27 status (Table III).

Changes in gelsolin concentration upon infliximab therapy

Following infliximab infusion, we did not observe any change in GSN serum levels. In this regard, the mean±SD values of GSN were 38660.42±23624.6 ng/ml immediately prior to infliximab infusion (time 0) and 36384.78±22808.01 ng/ml at the end of the infusion (time 120 minutes) (*p*=0.6641).

Discussion

GSN is a protein involved in cell locomotion and phagocytosis through binding to actin filaments (10). It has also been proposed that GSN could bind to factors such as fibronectin and fibrin, present in the inflamed joint space, in RA (11). Studies performed in patients as well as in mouse models have disclosed that GSN expression is reduced in rheumatoid synovial fibroblasts, leading to severe alterations in the cytoskeleton organisation (12). However, to our knowledge, there are no studies performed yet to evaluate how the chronic inflammation present in AS patients affects the levels of this protein.

Despite having an adequate control of the disease (mean BASDAI value at the time of the study: 2.94) and mild levels of inflammation, probably as the result of a long-term treatment with anti-TNF-α therapy, GSN levels were decreased in AS patients when compared to healthy controls. This is in keeping with previous reports that showed a decrease in GSN levels in situations of chronic inflammation or acute injury (10-12). In addition, when our series of AS patients was stratified according to sex, we observed that men had significantly lower levels of GSN than women. This could be correlated with the higher CV mortality found in men when compared with women.

With respect to the features of metabolic syndrome, we found a positive correlation between GSN levels and insulin resistance and a negative association with insulin sensitivity. However, the opposite results would be expected, since inflammation links to insulin resistance (32), and GSN would therefore act as an anti-inflammatory molecule. In fact, it was previously reported that GSN enhanced insulin secretion by pancreatic β cells (33). Therefore, it is possible that in our study we may be dealing with a paradoxical association that originates in a compensatory increase in GSN production aimed at reducing CV risk in the presence of insulin resistance. Nevertheless, whether

Table III. Differences in basal Gelsolin serum levels (time 0) according to categorical variables.

Variable	Category	Gelsolin: Mean±SD (ng/ml)	*p*
Sex	Men	32678.48 ± 15150.44	0.032
	Women	52618.27 ± 33708.24	
Arterial hypertension	Yes	34993.68 ± 15444.98	0.497
	No	41104.91 ± 27962.8	
Dyslipidaemia	Yes	37842.09 ± 25025.01	0.888
	No	39134.19 ± 23465.57	
Obesity	Yes	33189.43 ± 17526.06	0.680
	No	39268.3 ± 24393.75	
Current smoker	Yes	33554.82 ± 19317.73	0.309
	No	42564.69 ± 26350.32	
Hip involvement	Yes	42419.5 ± 29268.45	0.671
	No	37720.65 ± 22646.95	
Synovitis in other peripheral joints and peripheral enthesitis	Yes	35485.76 ± 23494.1	0.585
	No	40498.38 ± 24141.66	
Anterior uveitis	Yes	31724.04 ± 13302.18	0.431
	No	40394.51 ± 25486.52	
Syndesmophytes	Yes	34710.67 ± 11499.66	0.527
	No	40635.29 ± 27873.16	
HLA-B27	Positive	43670.11 ± 26795.77	0.167
	Negative	28643.07 ± 11775.01	

HLA: human leukocyte antigen; SD: standard deviation.

compensatory mechanisms associated with the prolonged use of anti-TNF-α therapy in these series of AS patients with low inflammatory burden at the time of the study may account for these results needs further elucidation.

Finally, when we evaluated the effect of a single infusion of anti-TNF-α infliximab on GSN serum levels in AS patients who were undergoing periodical treatment with this therapy, we did not observe any change. As pointed out before, a possible explanation for this steady level of GSN might be the low disease activity of our series of patients, since they had been receiving infliximab for a long period of time.

In conclusion, in AS patients undergoing periodical treatment with the anti-TNF-α monoclonal antibody-infliximab and low disease activity, GSN concentration is reduced when compared to controls. Potential association with some metabolic syndrome features seems to exist.

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