Apelin serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy

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Abstract

Objective

To determine whether disease activity, systemic inflammation and metabolic syndrome are potential determinants of circulating apelin in ankylosing spondylitis (AS) patients undergoing TNF-α antagonist-infliximab therapy.

Methods

We investigated apelin serum concentrations in a series of 30 non-diabetic AS patients without history of cardiovascular (CV) events that were treated with the TNF-α antagonist infliximab, immediately prior to an infliximab infusion. Correlations of apelin serum levels with disease activity, systemic inflammation and metabolic syndrome were assessed. Also, potential changes in apelin concentration following an infusion of the anti-TNF-α monoclonal antibody-infliximab were analysed.

Results

No significant correlation between apelin concentration and demographic features, inflammation, adiposity and metabolic syndrome features was seen. Neither differences were seen in basal apelin in different categorical variables associated to AS. Following infliximab infusion, a reduction of apelin serum levels was observed. In this regard, the median (interquartile range) values of apelin decreased from 0.99 (0.74–1.25) ng/ml immediately prior to infliximab infusion to 0.92 (0.72–1.39) ng/ml at the end of the infusion (time 120 minutes). However, the reduction in apelin serum levels following administration of the drug did not achieve statistical significance.

Conclusions

The present study shows that in non-diabetic patients with AS on treatment with infliximab apelin serum levels do not correlate with disease activity or metabolic syndrome. A single infusion of infliximab does not yield a significant change of apelin serum levels in AS patients.

Key words

Ankylosing spondylitis, atherosclerosis, inflammation, anti-TNF-α antibody-infliximab, apelin

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Received on November 15, 2012; accepted in revised form on January 15, 2013.

Funding: this study was supported by two grants from Fondo de Investigaciones Sanitarias PI06-0024 and PI09/00748 (Spain), and was partially supported by the RETICS Programme, RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII).

Competing interests: none declared.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease associated with increased cardiovascular (CV) mortality, 1.5–2 times higher than the normal population (1, 2). Alterations in surrogate markers of atherosclerosis, such as endothelial dysfunction and increased common carotid artery intima-media wall thickness, have been reported in AS patients (3-5). All these evidences support the presence of a process of accelerated atherosclerosis in patients with AS (6).

Traditional CV risk factors such as obesity and its related metabolic syndrome contribute to the increased CV morbidity and mortality in AS patients. However, the pro-inflammatory environment present in AS patients is propitious for the development of atherosclerotic lesions (2). Previously, we evaluated the pro-inflammatory adiponectin and resistin serum levels in non-diabetic AS patients on treatment with the anti-TNF-α monoclonal antibody-infliximab. We found a positive correlation of adiponectin with insulin sensitivity, suggesting that low circulating adiponectin concentrations may be involved in the pathogenesis of the CV disease in AS (7).

Anti-TNF-α therapy has been found to be effective in patients with AS and other spondyloarthropathies (8, 9, 10). Interestingly, a recent study disclosed that non-diabetic patients with AS, undergoing treatment with infliximab, experienced a rapid and dramatic reduction in serum insulin levels and a rapid improvement of insulin sensitivity after administration of this drug (11). Therefore, it is possible that TNF-α blockade might account for biological changes that may slow the progression of atherosclerosis in patients with AS. Because of that, an important step forward in our understanding of the effect of anti-TNF-α drugs in AS may be to establish potential changes in adipokines and biomarkers of endothelial cell activation following the administration of these biologic agents.

The hallmark of endothelial dysfunction, an early step in the atherogenic process, is impaired nitric oxide-mediated endothelial-dependent vasodilatation. This may be the result of diminished production or impaired activity of nitric oxide (12).

Recently, a new adipokine has entered the scene. Apelin is produced by many cell types, including endothelial cells and adipocytes. It is secreted as a pre-pro-peptide of 77 aminoacids, and is then cleaved into shorter isoforms, mainly apelin-12, 13 and 36. The shorter peptides exhibit the greater degrees of biological potency (13). Apelin has been associated with CV risk since it provokes the relaxation of smooth muscle cells of the artery wall, by promoting the release of nitric oxide. Low apelin levels have been associated with high LDL levels (14), and biomarkers of endothelial cell activation such as VCAM-1 and E-selectin were correlated to apelin levels (15).

Taking all these considerations together, in the present study we aimed to determine whether inflammation, metabolic syndrome or both of these characteristics are potential determinants of circulating apelin concentrations, and whether low apelin concentrations clustered with metabolic syndrome features in AS patients. We also assessed associations of circulating apelin concentrations with laboratory markers of inflammation and demographic characteristics of these patients. Moreover, we investigated whether infliximab administration altered circulating apelin concentrations in a 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 (16). They were treated by the same group of rheumatologists and were recruited from the Hospital Xeral-Calde, Lugo, Spain.

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 RA (17-19).

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 ischemic 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 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. However, since the criterion for initiation of infliximab therapy was severe disease refractory to NSAIDs, all of them had been treated with at least 3 NSAIDs prior to the onset of infliximab therapy (20).

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI- range of 0 to 10) (21) was evaluated in all patients at the time of the study. Clinical information on hip involvement, history of synovitis or enthesitis in other peripheral joints, history of anterior uveitis, presence of syndesmophytes and HLA-B27 status (typed by cell cytotoxicity) was assessed. Moreover, CRP- by a latex immunoturbidity method, 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. Also, information about CRP (by nephelometry) and ESR at the time of disease diagnosis was also reviewed.

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

Variable	
Mean age (years) ± SD	
At the time of study	50.5 ± 14.8
At the time of onset of symptoms	28.2 ± 10.4
Delay to the diagnosis (years) ± SD	11.5 ± 9.0
Men/Women	21/9
Mean disease duration (years) ± SD*	22.0 ± 13.2
History of classic cardiovascular risk factors	
Hypertension	12 (40.0%)
Dyslipidemia	11 (36.7%)
Obesity (BMI > 30 kg/m^2)	3 (10.0%)
Current smokers	13 (43.3%)
Mean blood pressure (mm Hg) ± SD*	
Systolic	123.2 ± 18.2
Diastolic	75.7 ± 12.5
Mean body mass index (kg/m^2) ± SD	26.7 ± 3.3
Mean BASDAI ± SD*	2.94 ± 2.11
Mean VAS ± SD*	31.1 ± 24.2
Hip involvement, n (%)	6 (20.0%)
Synovitis and/or enthesitis in other peripheral joints, n (%)	11 (36.7%)
Anterior uveitis, n (%)	6 (20.0%)
Syndesmophytes, n (%)	10 (33.3%)
Mean CRP (mg/l) ± SD**	
At the time of disease diagnosis	24.0 ± 33.4
At the time of study	6.2 ± 8.7
Mean ESR (mm/1st hour) ± SD***	
At the time of disease diagnosis	30.1 ± 28.2
At the time of study	19.0 ± 15.2
Mean cholesterol or triglycerides (mg/dl) ± SD*	
Total cholesterol	199.1 ± 30.6
HDL cholesterol	53.2 ± 12.8
LDL cholesterol	126.8 ± 26.5
Triglycerides	94.0 ± 56.7
Mean fasting serum glucose (mg/dl) ± SD*	92.8 ± 8.6
HLA-B27 positive (n=27)	20 (74.1%)

*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.

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, the mean BASDAI 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 one on the short term effect of infliximab therapy on insulin resistance in AS (11) was 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)÷22.5[7] (11). A commercial Apelin-12 Enzyme Immunoassay kit was used to measure serum apelin levels (Phoenix pharmaceuticals, EK-057-23; assay sensitivity=0.06 ng/ml; intra-and interassay coefficients of variation were <10% and <15%, respectively) (Phoenix pharmaceuticals, Burlingame, CA, USA). The Apelin-12 ELISA kit has 100% cross reactivity with human apelin-12, 13 and 36. Serum levels of Apelin were measured in samples obtained immediately prior to an infliximab infusion and 120 minutes later. Commercial ELISA kits (Linco Research, St. Charles, MO, USA) were used to measure total plasma adiponectin (Millipore, EZHADP-61K; assay sensitivity=0.5 ng/ml; intra- and interassay coefficients of variation were 3.3% and 5.5%, respectively), serum resistin (Millipore, EZHR-95K; assay sensitivity=0.16 ng/ml; intra- and interassay coefficients of variation were <5% and <7%, respectively) and serum leptin levels (Millipore, EZHL-80SK; assay sensitivity=0.135 ng/ml ± 2 SD; intra-and interassay coefficients of variation were 3.7% and 4%, respectively), according to the manufacturer's instructions, immediately prior to an infliximab infusion (7). Visfatin serum levels were determined by commercially available ELISA (Phoenix pharmaceuticals, EK-003-80; assay sensitivity = 2.68 ng/ml; intra- and interassay coefficients of variation were <10% and <15%, respectively) (Phoenix pharmaceuticals, Burlingame, CA, USA) according to the manufacturer's instructions.

Statistical analyses

Variables were expressed as mean ± standard deviation (SD), median (interquartile range – IQ) or percentages. Correlation between basal apelin 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 apelin concentrations were assessed by the Student's paired *t*-test for categorical variables. Differences in apelin levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U-test.

Apelin 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).

Table II. Partial correlation of basal serum apelin (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	r	*p*
Age at the onset of symptoms	-0.507	0.49
Disease duration*	-0.369	0.63
BMI*	0.349	0.65
Systolic blood pressure*	-0.487	0.51
Diastolic blood pressure*	0.377	0.62
BASDAI*	0.135	0.87
VAS*	0.437	0.56
ESR* (natural-log-transformed)	-0.431	0.57
CRP* (natural-log-transformed)	0.723	0.28
ESR** (natural-log-transformed)	0.617	0.38
CRP** (natural-log-transformed)	-0.130	0.87
Total cholesterol* (natural-log-transformed)	-0.256	0.74
HDL cholesterol* (natural-log-transformed)	0.458	0.54
LDL cholesterol* (natural-log-transformed)	0.033	0.97
Triglycerides* (natural-log-transformed)	0.560	0.44
Serum glucose* (natural-log-transformed)	-0.029	0.97
HOMA-IR*	-0.655	0.35
QUICKI*	-0.593	0.41
Resistin at time 0	0.563	0.44
Adiponectin at time 0	0.178	0.82
Leptin at time 0	-0.598	0.40
Visfatin at time 0	0.524	0.48

*At the time of the study. **At the time of disease diagnosis.
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.

Results

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

Apelin concentration 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, nor adiponectin, resistin, leptin, and visfatin concentrations (Table II). Likewise, no difference in apelin serum concentration between men and women was observed (Table III).

Relationships of apelin concentration with metabolic syndrome features other than adiposity

No significant correlation between apelin concentration with systolic or diastolic blood pressure, total cholesterol, HDL and LDL-cholesterol, triglycerides and glucose levels was observed (Table II). In keeping with these observations, no significant differences in apelin concentration were seen when patients were stratified according to the presence or absence of hypertension and dyslipidaemia (Table III). No significant correlation between apelin concentration and insulin sensitivity (QUICKI at the time of the study) was found (Table II).

Relationships of apelin concentration with other recorded baseline characteristics

Circulating apelin concentration did not correlate with disease duration, BASDAI and VAS spinal pain at the time of the study (Table II). Likewise, no difference in apelin concentration

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

Variable	Category	Apelin: Mean ± SD	*p*
Sex	Men	1.13 ± 0.66	
	Women	0.97 ± 0.38	0.52
Arterial hypertension	Yes	1.02 ± 0.22	
	No	1.12 ± 0.75	0.65
Dyslipidaemia	Yes	0.95 ± 0.25	
	No	1.16 ± 0.71	0.36
Obesity	Yes	0.82 ± 0.14	
	No	1.11 ± 0.61	0.43
Current smoker	Yes	1.23 ± 0.81	
	No	0.97 ± 0.33	0.24
Hip involvement	Yes	1.34 ± 1.12	
	No	1.02 ± 0.38	0.23
Synovitis and/or enthesitis in other peripheral joints	Yes	1.26 ± 0.84	
	No	0.98 ± 0.37	0.22
Anterior uveitis	Yes	0.92 ± 0.35	
	No	1.12 ± 0.64	0.46
Syndesmophytes	Yes	0.92 ± 0.22	
	No	1.16 ± 0.70	0.30
HLA-B27	Positive	1.02 ± 0.66	
	Negative	1.30 ± 0.45	0.30

HLA: human leukocyte antigen; SD: standard deviation.

Table IV. Differences in apelin serum concentration immediately before (time 0) and after (time 120 minutes) infliximab infusion.

Apelin	Basal (time 0)	Postinfusion (time 120)	*p*
(Median; IQ range)	0.99 (0.74-1.25)	0.92 (0.72-1.39)	0.36

IQ: interquartile.

was observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis and/or enthesitis in other peripheral joints 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 apelin concentration upon infliximab therapy

Following infliximab infusion, a reduction of apelin serum levels was observed. In this regard, the median (IQ range) values of apelin decreased from 0.99 (0.74–1.25) ng/ml immediately prior to infliximab infusion (time 0) to 0.92 (0.72–1.39) ng/ml at the end of the infusion (time 120 minutes). However, the reduction in apelin serum levels following administration of the drug did not achieve statistical significance (Table IV).

Discussion

Apelin is a new adipokine implicated in endothelium-dependent vasorelaxation by the activation of the eNOS pathway. High serum levels would therefore be expected to have an antiatherogenic role (22). The importance of apelin is exemplified by apelin-deficient mice, which suffer premature heart failure unless they are restored plasma apelin concentrations (23). Because of that, Goetze *et al*. had suggested that apelin could be a good marker for cardiovascular disease (24). In keeping with that, Li *et al*. compared the levels of apelin between stable angina patients and controls and found lower levels of this adipokine in the former group, suggesting that apelin may be involved in the pathophysiological process of coronary artery stenosis (25). Apelin has been shown to be expressed and released from adipocytes by factors such as insulin (26). This makes this peptide an attractive candidate to be studied in metabolic diseases such as type 2 diabetes. However, results regarding apelin levels in this disease are contradictory, since some authors found plasma apelin concentrations increased (27) while others found it low (28).

In the present study we assessed the possible association of apelin concentration and demographic features, inflammation, adiposity and metabolic syndrome features in a AS undergoing anti- TNF-α therapy. This fact may explain the low disease activity observed at the time of the study. However, no such a correlation was found, nor differences were seen in categorical variables associated to AS.

We also analysed the potential effect of the anti-TNF-α treatment on apelin concentration. We found that, after treatment infusion, apelin serum levels were reduced. The decrease, however, did not achieve statistical significance. In line with our results, Ferraz-Amaro *et al*. evaluated the levels of apelin and other adipokines in rheumatoid arthritis (RA) patients treated with anti-TNF-α, but they did not see any difference in its levels after 12 months of treatment. Neither did they find any correlation of adipokine concentrations with BMI or insulin resistance (29).

Interestingly, Di Franco *et al*. also studied apelin levels in a group of patients diagnosed with early stage RA. They found that this group had lower apelin levels than controls. They also studied if this level was affected after 12 months of treatment with DMARD therapy. They could see an appreciable decrease in apelin levels after this period. However, in keeping with our results, no statistically significant difference was observed (22).

In conclusion, the present study indicates that in non-diabetic patients with AS on treatment with infliximab apelin serum levels do not correlate with disease activity or metabolic syndrome. A single infusion of infliximab does not yield a significant reduction of apelin serum levels in AS patients.

Acknowledgements

The authors thank Mrs Susana Escandon and Isabel Castro-Fernandez, nurs-

es from the Rheumatology Outpatient Clinic, and Ms Pilar Ruiz, a nurse from the Haematology Division, and the members of the Biochemistry Department from Hospital Xeral-Calde, Lugo for their valuable help to undertake this study.

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