Osteoprotegerin correlates with disease activity and endothelial activation in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy

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Abstract

Objective

Osteoprotegerin (OPG) has been associated with increased risk and severity of atherosclerotic disease in the general population. Since ankylosing spondylitis (AS) is a chronic inflammatory disease associated with accelerated atherosclerosis, we aimed to assess whether OPG levels correlate with disease activity, systemic inflammation, metabolic syndrome, adipokines and biomarkers of endothelial cell activation in patients with AS undergoing TNF-α antagonist therapy.

Methods

We assessed OPG plasma concentration in 30 non-diabetic AS patients without cardiovascular disease undergoing TNF-α antagonist-infliximab therapy. OPG levels were measured immediately before and after an infliximab infusion. Correlations of OPG levels with disease activity, clinical characteristics, systemic inflammation, metabolic syndrome features, adipokines and biomarkers of endothelial activation were assessed. Changes in OPG concentration following an infusion of anti-TNF-α monoclonal antibody-infliximab were also analysed.

Results

We found a positive correlation between OPG levels and markers of disease activity such as BASDAI and VAS spinal pain (r=0.497, p=0.01; r=0.390; p=0.04, respectively). No differences in OPG levels according to specific clinical features of the disease were seen. An inverse correlation between OPG levels and total cholesterol and LDL-cholesterol was also found (r=-0.451; p=0.02 and r=-0.411; p=0.03, respectively). A correlation between OPG and asymmetric dimethylarginine, a biomarker of endothelial cell activation, was also disclosed (r=0.533; p=0.01). No correlation between OPG level and insulin resistance was observed. An infliximab infusion did not lead to a significant reduction in OPG levels.

Conclusion

OPG shows a correlation with markers of disease activity and endothelial activation in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy.

Key words

ankylosing spondylitis, atherosclerosis, inflammation, anti-TNF-α antibody-infliximab, osteoprotegerin

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Introduction

Traditional cardiovascular (CV) risk factors in combination with inflammation contribute to the increased CV morbidity and mortality observed in patients with ankylosing spondylitis (AS) (1). This is the result of a process of accelerated atherosclerosis (1, 2). Treatment with anti-tumor necrosis factor (TNF)-α agents has been found to be effective in patients with AS and other spondyloarthropathies (3-5). It has been postulated that TNF-α blockade may account for biological changes that may slow the progression of atherosclerosis in these patients (6). Therefore, the analysis of the potential influence of treatment with anti-TNF-α drugs on disease activity, systemic inflammation, metabolic syndrome, adipokines and biomarkers of endothelial cell activation may be of potential interest to improve our understanding of the effect of these biologic agents on the mechanism associated with atherosclerosis in AS patients.

Over the last three years our group has been engaged in the study of the biologic effects of anti-TNF-α monoclonal antibody-infliximab therapy in a series of non-diabetic AS patients. We first observed that infliximab treatment reduced serum insulin levels and improved insulin sensitivity (7). More recently, we observed that infusion of this drug led to a significant reduction of retinol-binding protein-4 (RBP-4), an emerging cardiometabolic risk factor linked with insulin resistance (8). To further establish potential beneficial effects of the use of anti-TNF-α therapy on the metabolic syndrome associated with AS, we also studied adipokine serum levels in these patients. We found a positive correlation between adiponectin and insulin sensitivity, suggesting that low circulating adiponectin concentrations may be involved in the pathogenesis of the CV disease in AS (9). We also disclosed a significant positive correlation of visfatin with insulin resistance (10). However, we could not find an association of apelin with disease activity or metabolic syndrome (11). Nevertheless, we disclosed a correlation between serum levels of the peptide ghrelin and insulin resistance (12).

Interestingly, we also disclosed that asymmetric dimethylarginine (ADMA) serum levels were associated with some features of metabolic syndrome such as sex and hypertension in patients with AS (13). It was of potential relevance as ADMA is a biomarker of endothelial activation, an early step in the atherogenesis process. Anti-TNF-α infliximab infusion led to a dramatic reduction of angiopoietin-2 (Angpt-2), another marker of endothelial cell activation that is involved in angiogenesis making the endothelium responsive to inflammatory cytokines (14). In line with these results, we also found a positive correlation between serum levels of Angpt-2 and osteopontin (OPN), another biomarker of atherosclerosis. Furthermore, we also disclosed a statistically significant reduction in OPN concentration after a single anti-TNF-α- infliximab infusion (15).

Since inflammation plays an important role in the development of atherosclerosis, the study of molecules involved in both processes is of main importance to establish potential predictors of CV disease in patients with chronic rheumatic diseases. One of these molecules is osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor super-family implicated in bone remodeling that acts as a decoy receptor for the receptor activator of nuclear factor-κB ligand (RANKL) (16, 17). OPG decreases binding of RANKL to its receptor, RANK. Besides its implication in osteoporosis, the OPG/RANKL/RANK axis has been found to be involved in atherosclerosis (16, 17). OPG also acts as a soluble neutralising receptor for another member of the TNF superfamily, TNF-related apoptosis inducing ligand (TRAIL), a molecule with anti-inflammatory and anti-atherosclerotic properties (18-20). OPG is produced by most human tissues and cells that include not only osteoblasts but also endothelial and vascular smooth muscle cells. OPG can upregulate endothelial adhesion molecule production (21) and increase leukocyte adhesion to endothelial cells (22). In addition, OPG is also present in atherosclerotic plaques and this molecule has been associated with the increased risk and severity of

coronary artery disease (23-25), incident coronary artery disease (26, 27), cerebrovascular disease (28) and peripheral vascular disease (29).

Taking these considerations together, in the present study we aimed to evaluate whether circulating OPG concentrations correlate with disease activity and clinical characteristics in a series of non-diabetic AS patients undergoing TNF-α antagonist-infliximab-therapy. We also aimed to determine potential correlation between OPG levels and systemic inflammation, metabolic syndrome, adipokines and biomarkers of endothelial activation in these patients. In addition we studied the effect of an infliximab infusion on levels of circulating OPG.

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 (30). They were treated by the same group of rheumatologists and were recruited from the Hospital Lucus Augusti, 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 adipokines and biomarkers of endothelial cell activation in patients with rheumatoid arthritis (RA) (31-33).

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. Patients were considered to have dyslipidaemia if they had hypercholesterolaemia and/or hypertriglyceridaemia (defined as diagnosis of hypercholesterolaemia or hypertriglyceridaemia by the patients' family physicians, or total cholesterol and/or triglyceride levels in fasting plasma were >220 mg/dl and 150 mg/dl, respectively). Obesity was defined if body mass index (BMI) (calculated as weight in kilograms divided by height in squared metres) 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 (34), 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 therapy.

A clinical index of disease activity (Bath Ankylosing Spondylitis Disease Activity Index- BASDAI- range of 0 to 10) (35) 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 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 this 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 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 erythrocyte sedimentation rate [ESR] (Westergren), C-reactive protein [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 (7).

Osteoprotegerin (OPG) ELISA

Human OPG plasma levels were determined by ELISA in samples obtained at time 0 and 120 minutes after infliximab infusion. Briefly, 96-well microplates were precoated with anti-human OPG antibody (Peprotech). Recombinant human OPG (Peprotech) was used to prepare the standard curve. The standard dilution series ranged from 0.313 to 20 ng/ml. First, 50 μl of each standard or sample was added to the appropriate wells and incubated for 3 hours at room temperature. After discarding the solution and washing 4 times, 50 μl of prepared biotinylated anti-human OPG antibody (Peprotech) was added to each well and incubated for 1 hour. After washing away unbound biotinylated antibody, 50 μl of horseradish peroxidase (HRP)-conjugated avidin (eBioscience) was pipetted into the wells and incubated for 30 minutes. Finally, plates were developed with ABTS Liq-

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*	21.9 ± 13.16
History of classic cardiovascular risk factors	
Hypertension (n=30)	12 (40)
Dyslipidaemia (n=30)	11 (36.67)
Obesity (BMI > 30 kg/m2) (n=30)	3 (10.00)
Current smokers (n=30)	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 ±SD*	31.13 ± 24.23
Hip involvement, n (%)(n=30)	6 (20)
Synovitis and/or enthesitis in other peripheral joints, n (%)(n=27)	11 (36.67)
Anterior uveitis, n (%)(n=30)	6 (20.00)
Syndesmophytes, n (%)(n=30)	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.

uid Substrate (Peprotech) and read at 405 and 600 nm (as reference wavelength).

Other ELISA determinations

Total plasma adiponectin, serum resistin, leptin, visfatin, apelin, Angpt-2, ADMA, ghrelin, OPN and RBP-4 levels immediately prior to and after an infliximab infusion were determined by ELISA as previously described (8-15).

Statistical analyses

Variables were expressed as mean ± SD or percentages. Correlation between basal OPG at time 0 with selected continuous variables was performed adjusting for 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 plasma OPG concentrations were assessed by the Student's paired *t*-test. Differences in OPG levels between men and women and patients with hypertension or not were assessed by Mann-Whitney U test.

OPG plasma levels before (at time 0) and postinfusion (at time 120 minutes) 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

Relationships of osteoprotegerin concentration with disease activity and clinical features

Circulating OPG concentration did not correlate with disease duration. Nevertheless, we observed a partial correlation between OPG plasma levels a disease activity. In this regard, a significant correlation between OPG and BASDAI (r=0.497; *p*=0.01) and VAS spinal pain (r=0.390; *p*=0.04) at the time of the study was disclosed (Table II). Nevertheless, no differences in OPG concentration were observed when patients with a history of anterior uveitis, presence of syndesmophytes, hip involvement or synovitis in other peripheral joints and peripheral enthesitis were compared with the remaining patients who did not exhibit these clinical characteristics (Table III). Although OPG levels were higher in HLA-B27 positive (2.09±1.09 ng/ml) than in HLA-B27 negative patients (1.34±0.48 ng/ml) the difference was out of the range of significance (*p*= 0.093).

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

OPG plasma levels did not show significant correlation with the age at the onset of symptoms, BMI, and CRP and ESR at the time of the study and at the time of disease diagnosis (Table II). In addition, we did not observe any association with resistin, adiponectin, leptin, visfatin and apelin levels (Table II). Neither did we observe significant differences in OPG plasma levels when AS patients were stratified according to sex (Table III).

Relationships of osteoprotegerin concentration with metabolic syndrome features other than adiposity

OPG plasma levels inversely correlated with total cholesterol (r=-0.451; *p*=0.02) and LDL-cholesterol (r=-0.411; *p*=0.03). However, we did not observe any statistically significant correlation between OPG plasma levels with systolic or diastolic blood pressure, HDL-cholesterol, triglycerides, serum glucose levels, insulin sensitivity

Table II. Partial correlation of plasma OPG 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 (dyslipidaemia, smoking, obesity, hypertension) in 30 patients with ankylosing spondylitis.

Variable	OPG (time 0) r	*p*-value
Age at the onset of symptoms	-0.066	0.74
Disease duration*	0.103	0.61
BMI*	-0.173	0.39
Systolic blood pressure*	0.123	0.54
Diastolic blood pressure*	-0.034	0.87
BASDAI*	**0.497**	**0.01**
VAS spinal pain*	**0.390**	**0.04**
ESR* (natural-log-transformed)	0.071	0.73
CRP* (natural-log-transformed)	0.207	0.30
ESR** (natural-log-transformed)	-0.228	0.25
CRP** (natural-log-transformed)	0.015	0.94
Total cholesterol* (natural-log-transformed)	**-0.451**	**0.02**
HDL cholesterol* (natural-log-transformed)	-0.225	0.26
LDL cholesterol* (natural-log-transformed)	**-0.411**	**0.03**
Triglycerides* (natural-log-transformed)	-0.095	0.64
Serum glucose* (natural-log-transformed)	-0.293	0.14
HOMA-IR at time 0*	0.231	0.25
QUICKI at time 0*	-0.225	0.26
Resistin at time 0	-0.076	0.74
Adiponectin at time 0	-0.191	0.35
Leptin at time 0	-0.158	0.44
Visfatin at time 0	0.293	0.14
Angpt-2 at time 0	0.180	0.37
Apelin at time 0	0.155	0.44
ADMA at time 0	**0.533**	**0.01**
Ghrelin at time 0	-0.042	0.84
OPN at time 0	-0.055	0.78
RBP-4 at time 0	0.005	0.98

*At the time of the study. **At the time of disease diagnosis.
ADMA: Asymetric dimethylarginine; Angpt-2: Angiopoietin 2; 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; OPN: Osteopontin; QUICKI: Quantitative insulin sensitivity check index; RBP-4: Retinol-binding protein-4; VAS: Visual analogue scale.
Significant results are highlighted in bold.

(QUICKI) or insulin resistance (HOMA-IR) (Table II). Likewise, we did not find any correlation between OPG concentration and metabolic syndrome-associated biomarkers such as ghrelin or RBP-4 (Table II). Besides, no significant differences in OPG concentration were seen when patients were stratified according to the presence or absence of arterial hypertension (Table III).

Relationship of osteoprotegerin plasma levels with biomarkers of endothelial cell activation and atherosclerosis

We found a positive correlation between OPG and ADMA concentration (r=0.533; p=0.01). However, no correlation of OPG levels with Angpt-2 or OPN, two biomarkers of atherosclerosis, was found.

Changes in osteoprotegerin concentration upon infliximab therapy

Following an infliximab infusion, we observed a decrease in OPG plasma levels. In this regard, the mean ± SD values of OPG found immediately prior to infliximab infusion (1.79±1.02 ng/ml) were higher than those found at the end of the infusion (and 1.58±0.86 ng/ml). However, the difference did not reach statistical significance (p=0.215).

Discussion

The present study shows an independent relationship between OPG concentrations and endothelial activation in AS. Furthermore, OPG concentrations were linked to disease activity, and paradoxically related to low lipid concentrations as was also found in RA (36).

Our results indicate for the first time that in patients with AS undergoing anti-TNF-α therapy, OPG concentrations are associated with ADMA, a biomarker of endothelial cell activation, independent of traditional CV risk factors. This observation is in keeping with results reported by Berg *et al*., who demonstrated elevated OPG and ADMA levels in a series of 145 AS patients when compared with 125 controls (37). ADMA inhibits NO synthase, and thus contributes to endothelial dysfunction, an early step in the atherogenic process (13).

OPG was reported to be an independent risk factor for the progression of atherosclerosis and onset of CV disease in a population based study (27). In that series OPG was related to markers of endothelial activation, systemic inflammation and major proinflammatory conditions such as chronic infection or smoking (27). Interestingly, despite having good control of the disease (BASDAI<4) and mild elevation of laboratory markers of inflammation at the time of the study, we observed a correlation between disease activity and OPG concentrations in our series of AS undergoing infliximab therapy. In this regard, both BASDAI and VAS spinal pain showed a significant correlation with OPG levels. However, we could not find a correlation between CRP or ESR and OPG levels in our study. It is possible that this lack of association between OPG and markers of systemic inflammation may be the result of reduction of the inflammatory burden mediated by the prolonged use of infliximab as the median duration of infliximab was almost 2 years and the mean BASDAI only 2.94 in our series of AS patients.

In our study we also observed a negative correlation between OPG concentrations and total cholesterol and LDL cholesterol. This inverse correlation between OPG and lipid parameters associated with increased risk of CV disease appears to be somehow paradoxical. Nevertheless, similar findings were described in individuals without rheumatic diseases (38). In addition, a recent study has also shown a favourable relationship between OPG

Table III. Differences in basal OPG plasma levels (time 0) according to categorical variables.

Variable	Category	OPG: Mean ± SD (ng/ml)	*p*-value
Sex	Men	1.80 ± 1.02	0.942
	Women	1.77 ± 1.08	
Arterial hypertension	Yes	2.18 ± 1.10	0.091
	No	1.54 ± 0.90	
Dyslipidaemia	Yes	1.97 ± 1.21	0.480
	No	1.69 ± 0.91	
Obesity	Yes	1.29 ± 0.57	0.380
	No	1.85 ± 1.05	
Current smoker	Yes	2.02 ± 1.13	0.285
	No	1.61 ± 0.91	
Hip involvement	Yes	2.05 ± 0.87	0.492
	No	1.73 ± 1.06	
Synovitis in other peripheral joints and peripheral enthesitis	Yes	1.82 ± 1.04	0.900
	No	1.77 ± 1.03	
Anterior uveitis	Yes	1.59 ± 1.07	0.588
	No	1.84 ± 1.02	
Syndesmophytes	Yes	1.63 ± 1.03	0.552
	No	1.87 ± 1.03	
HLA-B27	Positive	2.09 ± 1.09	0.093
	Negative	1.34 ± 0.48	

HLA: human leukocyte antigen; SD: standard deviation.

concentrations and lipid parameters in patients with RA (36). These apparently contradictory results in patients with chronic rheumatic diseases might speak in favour of potential compensatory mechanisms mediated by the OPG molecule. However, AS patients in our series and in those with RA reported by Dessein *et al*. (36) were undergoing anti-TNF-α therapy, and it is well known that prolonged infliximab use is associated with qualitative and quantitative changes in lipid profile of patients with rheumatic diseases (39).

Recent evidence indicates that in RA patients with active disease levels of cholesterol and LDL-cholesterol are unexpectedly low (40, 41). This is the result of inflammation as improvement of the disease is associated with elevation of lipids, including total cholesterol and LDL-cholesterol (40, 41). In the present study there was an association between endothelial cell activation and OPG in AS since a high positive correlation (r= 0.533) between ADMA and OPG was observed. In this regard, although patients from our series had low disease activity probably because of prolonged TNF-α antagonist therapy, the levels of CRP were still above the normal range as the mean value was 6.24 mg/l. With respect to this, in a large cohort of apparently healthy women, values of CRP greater than 3 mg/l were associated with high risk of future CV events (42). Therefore, despite having low disease activity, to a greater or less degree a chronic inflammatory state was present is this series of patients with AS.

Finally, Dessein *et al*. observed that in long-standing RA patients with severe disease undergoing infliximab therapy, who persisted with active disease despite use of this biologic agent, there was a significant reduction of OPG within 2 hours after a repeat infusion (36). In contrast, in our present series of AS the decrease of OPG concentration following an infliximab infusion was not significant. We feel that the lack of significant reduction of OPG upon infusion of this biologic agent in our series of AS patients was due to the low disease activity and low inflammatory burden observed at the time of the study.

In conclusion the present results add substantially to the increasing evidence that OPG is a valuable CV disease risk biomarker and may well be proatherogenic in chronic inflammatory rheumatic diseases. OPG concentrations correlated with endothelial activation and disease activity in patients with AS independent of systemic inflammation and classic cardiovascular risk factors.

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