Anti-IL-6 therapy reduces leptin serum levels in patients with rheumatoid arthritis

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Abstract

Objective

Leptin is an adipokine that participates in the regulation of the immune and inflammatory response. Chronic systemic inflammation contributes to the development of cardiovascular (CV) disease in rheumatoid arthritis (RA). In this study, we aimed to assess the short-term effect of the anti-IL-6 receptor tocilizumab (TCZ) administration on circulating leptin concentrations in patients with RA, as well as the potential association of leptin with CV risk factors and demographic and clinical characteristics of these patients.

Methods

We recruited 50 consecutive non-diabetic patients with RA undergoing periodic treatment with TCZ. Leptin serum levels were determined by a commercial immunoassay kit in samples obtained immediately prior to (pre-infusion) and 60 minutes after the end of a TCZ intravenous infusion (post-infusion).

Results

A significant reduction of leptin levels was observed following the TCZ infusion (9.24±7.98 ng/mL vs. 7.92±7.32 ng/mL, pre- and post-infusion, respectively, p=0.002). Additionally, there was a strong positive correlation between body mass index of RA patients and basal levels of leptin (r=0.56; p=0.0001). Moreover, high basal levels of leptin in RA patients were associated with female sex (p=0.006), obesity (p<0.001) and rheumatoid factor negative status (p=0.006).

Conclusion

Our study disclosed a short-term effect of anti-IL-6 therapy on leptin serum levels in RA patients. Decreased leptin levels may explain the beneficial effect of anti-IL-6 blockade on CV disease associated to RA.

Key words

rheumatoid arthritis, inflammation, cardiovascular disease, leptin, anti-IL-6 receptor tocilizumab

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Introduction

Increased mortality has been observed in rheumatoid arthritis (RA) patients as a consequence of endothelial damage and accelerated atherosclerosis leading to high incidence of cardiovascular (CV) disease (1-3). The presence of traditional CV risk factors (most of them clustered under the term 'metabolic syndrome' (MetS)), the genetic component, immune dysregulation and chronic systemic inflammation play a critical role in the development of CV disease (3).

Growing evidence supports the relevant role of leptin as a key molecule involved in autoimmune diseases such as RA (4-6). This adipokine is mainly synthesised by adipose tissue and their surrounding macrophages. Although the best-known effect of leptin is on appetite regulation, immunomodulatory functions are also important (5, 6). Leptin levels have been shown increased in conditions of inflammation, as a result of enhanced production mediated by cytokines such as interleukin 6 (IL-6) (5). Consequently, leptin acts as a proinflammatory cytokine (6). Moreover, several studies have suggested that elevated leptin levels predict the occurrence of MetS and CV disease (4, 7).

IL-6 constitutes a main clinical target in RA treatment (8). Accordingly, tocilizumab (TCZ), a humanised antihuman IL-6 receptor monoclonal antibody, has been effectively used in the treatment of RA (8). In this sense, in a previous study of our group performed in RA patients, a rapid beneficial effect of intravenous TCZ on insulin resistance, a mechanism involved in the development of MetS and CV disease, was observed (9).

Consequently, the purpose of the present study was to investigate the short-term effect of intravenous TCZ administration on circulating leptin serum levels in 50 patients with RA that were on periodical treatment with this biologic agent. We also aimed to assess the potential association of leptin with CV risk factors and demographic and clinical characteristics of these patients.

Patients and methods

Patient population

A series of 50 consecutive non-diabetic patients diagnosed with RA according to the 2010 American College of Rheumatology classification criteria (10) were included in this study. The main characteristics of this series of patients have previously been described (9).

These patients were on periodical treatment with TCZ (RoActemra®, Roche Laboratories, Spain), which was administered intravenously at variable doses ranging between 4 and 8 mg/kg every 4-6 weeks. This series of patients included long-standing RA patients. In all cases, TCZ was started because of severe disease refractory to therapy with disease-modifying anti-rheumatic drugs and in many cases, to other biologic agents. These patients were recruited from the Rheumatology outpatient clinics of Hospital de La Princesa (Madrid, Spain) and Hospital de Sierrallana (Cantabria, Spain).

Patients with diabetes, fasting plasma glucose levels >125 mg/dl, currently taking anti-diabetic oral drugs, with chronic kidney disease (serum creatinine ≥1.3 mg/dl or glomerular filtration rate <60 ml/min) or with body mass index (BMI) ≥35 (kg/m^2) were excluded.

The local institutional ethics committee of every participant centre approved the study protocol and it was in accordance with the principles of the Declaration of Helsinki. All participants gave their written informed consent to participate in this study before their inclusion. This study has not been supported by any pharmaceutical company.

Study protocol

A comparison of the effect of TCZ administration in each patient (before and after) was performed.

In all cases, TCZ was given as an intravenous infusion in a saline solution over 60 minutes. The first sample was taken immediately prior to the TCZ infusion (pre-infusion), and the second sample 60 minutes after finishing TCZ infusion (post-infusion). Simultaneously, vital signs were also registered. All measurements were made in the fasting state.

A commercial immunoassay kit was used to measure human leptin serum levels (Human Leptin ELISA Kit, EZHL-80SK, EMD Millipore).

Routine laboratory parameters were as-

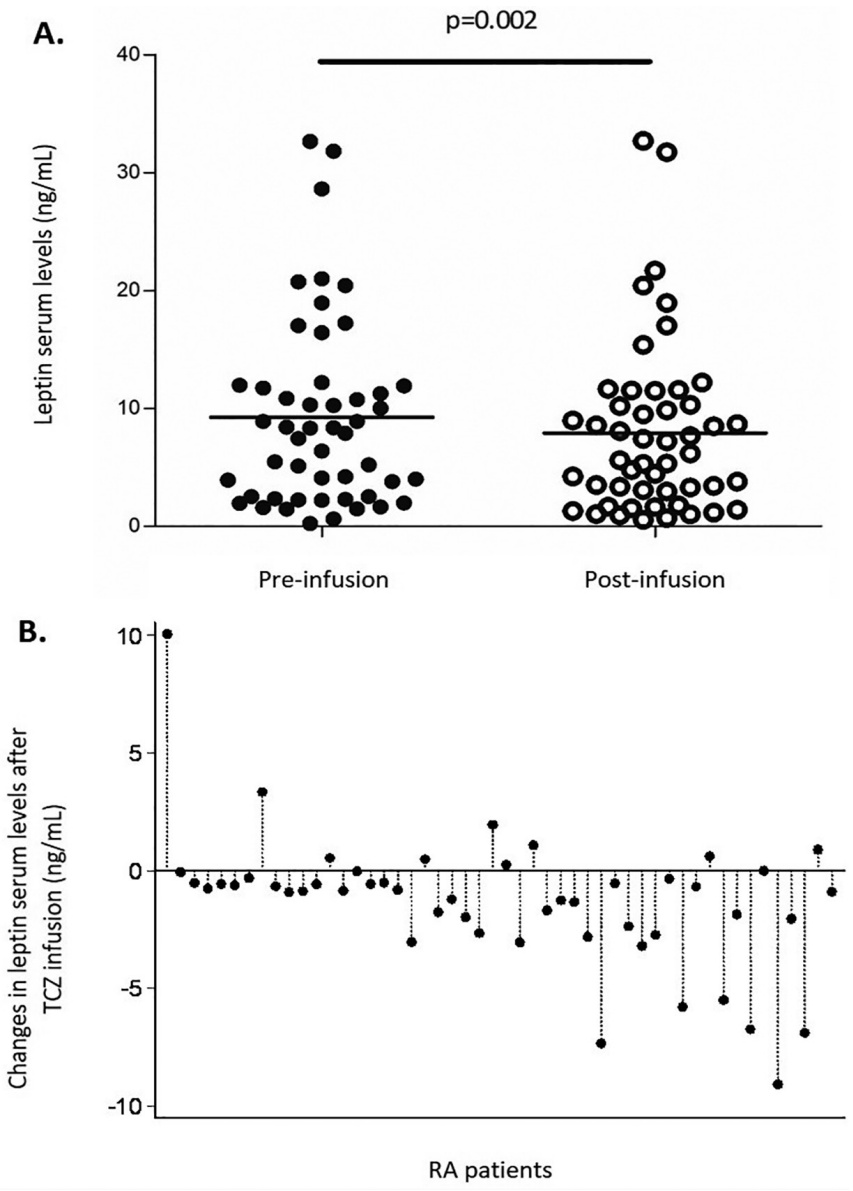

Fig. 1. Leptin serum levels following TCZ infusion.
A: Significant reduction of leptin serum levels following TCZ infusion. Horizontal bars indicate mean value of each study group.
B: Changes in leptin serum levels in each individual after TCZ infusion. Patients are ordered from the lowest to the highest pre-infusion leptin basal levels.

sessed in blood samples taken prior to TCZ infusion as previously described (9).

Statistical analyses

Results were reported as mean ± standard deviation (SD). For the comparison of leptin serum levels before and after TCZ infusion, paired Student's *t*-test was used. Relationship of leptin prior to TCZ (basal) with selected continuous and categorical variables was performed adjusting by age at the time of the study, sex and classic CV risk factors via estimation of the Pearson's partial correlation coefficient (r) and lineal regression, respectively. Statistically significant differences were considered at $p<0.05$. Statistical analysis was performed using the software STATA 12/SE (StataCorp, College Station, TX, USA).

Results

Changes in leptin concentrations upon TCZ therapy

A significant reduction of leptin levels was observed following the TCZ infusion in RA patients. Leptin levels decreased from 9.24±7.98 ng/mL immediately prior to TCZ infusion to 7.92±7.32 ng/mL after the infusion ($p=0.002$) (Fig. 1A). Moreover, higher leptin basal levels were associated with a greater decrease in leptin levels after TCZ treatment (Fig. 1B).

Relationship of leptin concentrations with demographic features and CV risk factors

We observed that women showed significantly higher leptin basal concentrations when compared to men (10.30±8.22 ng/mL *vs*. 3.66±3.02 ng/mL, respectively, $p=0.006$) (Table I). Likewise, patients with obesity exhibited increased basal levels of leptin (16.90±8.87 ng/mL) with respect to those non-obese (7.33±6.56 ng/mL) ($p<0.001$) (Table I). Accordingly, a strong significant positive correlation between leptin basal levels and BMI was disclosed (r=0.56; $p=0.0001$) (Table II).

Relationship of leptin concentration with disease activity and clinical features

A significant difference in leptin basal levels between RF positive (7.60±7.20 ng/mL) and negative (12.78 ± 8.95 ng/mL) patients was disclosed ($p=0.006$) (Table I).

Discussion

CV disease is the most relevant comorbidity in patients with RA (1-3). The great contribution of MetS to the CV morbidity and mortality in these patients has deserved great attention in the last years (3). Accordingly, our group previously reported a rapid beneficial effect of a single administration of intravenous TCZ on insulin resistance, one of the CV risk factors clustered in MetS, in patients with RA (9).

The adipokine leptin was suggested to be a key molecule in the pathogenesis of RA (4-6). It has also been associated with inflammation, MetS and CV disease (4-7). Therefore, the purpose of this study was to assess the short-term effect of IL-6 receptor blockade by intravenous TCZ administration on leptin serum levels in RA patients.

Table I. Differences in leptin basal levels according to categorical variables, adjusting for age at the time of the study, sex and classic cardiovascular risk factors.

Variable	Category (n)	Leptin (ng/mL)	
		Mean ± SD	*p*-value
Sex	Men (8)	3.66 ± 3.02	**0.006**
	Women (42)	10.30 ± 8.22	
RF	Yes (34)	7.60 ± 7.20	**0.006**
	No (15)	12.78 ± 8.95	
Anti-CCP	Yes (24)	10.27 ± 8.70	0.88
	No (11)	9.93 ± 5.74	
Obesity	Yes (10)	16.90 ± 8.87	**<0.001**
	No (40)	7.33 ± 6.56	
Arterial hypertension	Yes (22)	10.57 ± 9.10	0.67
	No (28)	8.20 ± 6.98	
Dyslipidaemia	Yes (14)	9.32 ± 7.21	0.83
	No (36)	9.21 ± 8.36	

SD: standard deviation; RF: rheumatoid factor; Anti-CCP: Anti-cyclic citrullinated peptide antibodies. Significant results are highlighted in bold.

Table II. Partial correlation of leptin basal levels with selected continuous variables, adjusting for age at the time of the study, sex and classic cardiovascular risk factors.

Variable	Leptin	
	r	*p*-value
Age at the onset of symptoms	0.19	0.21
Age at the time of the study	0.18	0.22
Disease duration	-0.19	0.22
Disease activity		
DAS28-ESR	-0.04	0.81
DAS28-CRP	-0.12	0.46
Swollen Joints	-0.06	0.68
Tender Joints	-0.01	0.95
VAS patient	-0.05	0.74
CRP	-0.04	0.80
ESR	0.06	0.72
Metabolic syndrome		
BMI	0.56	**0.0001**
Glucose	0.03	0.86
Insulin	0.24	0.12
Insulin/Glucose Index	0.19	0.22
HOMA-IR	0.24	0.11
QUICKI	-0.21	0.16
Total cholesterol	0.28	0.06
HDL-cholesterol	-0.04	0.82
LDL-cholesterol	0.15	0.35
Triglycerides	-0.07	0.65
C-peptide	0.27	0.08
Systolic blood pressure	-0.03	0.82
Diastolic blood pressure	-0.21	0.16

DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; VAS: visual analogue scale; BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index; HDL: high-density lipoprotein; LDL: low-density lipoprotein. Significant results are highlighted in bold.

We showed for the first time that TCZ infusion yields a rapid reduction of leptin serum levels in patients with RA. This makes sense considering that the production of leptin is stimulated by IL-6 (5). In our study the changes in leptin levels following a single intravenous TCZ infusion were dependent on the basal levels of leptin. The higher the basal levels were, the greater the decrease in leptin levels after a TCZ infusion was.

Our study intended to determine the short-term effect of anti-IL-6 receptor TCZ on leptin levels. However, no significant long-term modifications of leptin serum levels in patients with RA treated with TCZ were reported by Hoffman *et al*. and Fioravanti *et al*. (11, 12). This apparent discrepancy with our results may be related to the bioavailability of the biologic agent. In this regard, Hoffman *et al*. and Fioravanti *et al*. performed long-term analyses (at 4 and 6 months, respectively) of patients treated with 8 mg/kg of intravenous TCZ every 4 weeks (11, 12), while our study evaluated the short-term, almost immediate, effect of TCZ on leptin serum levels measured 1 hour after the intravenous infusion. It is possible that the half-life of this biologic agent (11-14 days) may explain that the effect of TCZ on leptin may be not significant 4 weeks after the infusion. However, in a previous study performed by our group, no significant changes in leptin concentrations were seen when the short-term effect of the intravenous anti-TNF-infliximab treatment on leptin serum levels in RA patients with active and refractory disease was evaluated (13). Accordingly, it is possible that the blockade of IL-6 signalling pathway might be more effective than TNF blockade to modulate metabolic effects in RA patients.

An increase of leptin in women when compared with men was found in our patients. These observations are in keeping with other studies of our group that also reached the same results in patients with psoriasis and ankylosing spondylitis (14, 15). Furthermore, in accordance with the relationship of leptin with MetS (4), in our study leptin serum levels were increased in obese patients compared to non-obese patients. This link between leptin and obesity was further supported by the statistically significant positive correlation of leptin serum levels with BMI. Therefore, our study confirms previous reports showing that BMI may be an important determinant of leptin levels in patients with RA and other inflammatory diseases such as psoriasis (14).

Finally, we found an association of high serum levels of leptin with RF negative status. However, further analysis should be needed to confirm this relationship.

In conclusion, our study disclosed for the first time a short-term effect of anti-IL-6 therapy on leptin serum levels in RA patients. The decreased leptin serum levels observed following an intravenous TCZ infusion may support a beneficial effect of anti-IL-6 blockade on CV disease associated with RA.

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