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Circulating osteoprotegerin is increased in the metabolic syndrome and associates with subclinical atherosclerosis and coronary arterial calcification

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ABSTRACT

Context: The relationship between osteoprotegerin (OPG) a glycoprotein related to bone metabolism and the metabolic syndrome (MS) has not been established.

Objective: The aim of this study is to evaluate OPG concentration in patients with MS and its association with subclinical atherosclerosis and coronary arterial calcification (CAC).

Materials/methods: The study included 238 asymptomatic patients. MS was diagnosed according to the NCEP/ATPIII guidelines. OPG was measured by ELISA. All subjects underwent ultrasonography of the common carotid arteries to measure intima-media thickness (IMT) and evaluate the presence of atheroma plaques. In a subgroup ($n = 39$) CAC was quantified by ECG-triggered cardiac computed tomography. Adipose tissue was excised from 25 patients and OPG expression by RT-PCR and immunohistochemistry was studied.

Results: Patients with the MS ($n = 60$) had higher OPG than patients without ($n = 178$) ($p < 0.05$). OPG correlated with IMT ($r = 0.2$, $p = 0.005$) and patients with atheroma plaques had higher OPG ($p = 0.008$) and also those with coronary artery calcification ($p < 0.05$).
OPG expression was confirmed in adipose tissue ($n = 12$) and the expression was significantly higher in patients with MS than in those without ($p = 0.003$).

Conclusions: This study shows that OPG may potentially be a biomarker for cardiovascular risk/damage in the MS and identifies adipose tissue as a potential source of OPG.

Introduction

Osteoprotegerin (OPG) is a soluble glycoprotein member of the tumor necrosis factor (TNF) receptor superfamily, originally discovered as an inhibitor of osteoclastogenesis. Biochemically, OPG is a basic secretory glycoprotein composed of 380 aminoacids and seven structural domains which exists as a more active monomeric form (~60-kDa) and a homodimeric form [1].

OPG is part of the OPG/receptor activator of NF-κB ligand (RANKL)/receptor activator of NF-κB (RANK) pathway. The RANKL/OPG/RANK axis has been shown to regulate bone remodeling. RANKL–RANK interaction leads to the transcription of specific genes required for osteoclast differentiation. OPG acts as a soluble decoy substrate to the receptor activator of RANKL and competes with RANK, inhibiting RANKL–RANK interactions and thus proliferation and differentiation of osteoclasts and consequently bone resorption [2].

In addition to being central to regulating RANK–RANKL interactions in bone metabolism, several studies suggest that there is a potential role of OPG in mediating cardiovascular damage [1,3]. In vitro studies indicate that OPG is expressed in cells involved in atheroma plaque development and progression, such as arterial smooth muscle cells [4], endothelial cells [5] and megakaryocytes [6]. Moreover, OPG expression is enhanced in explanted human carotid atherosclerotic plaques [7].

Human studies show a positive relationship between circulating OPG, vascular damage and cardiovascular disease. Indeed, elevated serum OPG levels have been found associated with atherosclerosis [8]

Abbreviations: ΔCt, Delta Theshold Cycle; BMI, Body Mass Index; CAC, Coronary Artery Calcium; CT, Computed Tomography; Ct, Theshold Cycle; ECG, Electrocardiogram; FOV, Field Of View; HDL, High Density Lipoprotein; HU, Hounsfield Units; IMT, Intima-Media Thickness; LDL, Low Density Lipoprotein; MDRD, Modification of Diet in Renal Disease; MS, Metabolic syndrome; NCEP-ATPIII, National Cholesterol Education Program-Adult Treatment Panel III; OPG, Osteoprotegerin; RANK, Receptor Activator of Nuclear Factor-κappa βeta; RANKL, Receptor Activator of Nuclear Factor-κappa βeta Ligand; RT-PCR, Real-Time reverse transcriptase-Polymerase Chain Reaction; PCR, Polymerase Chain Reaction; SD, Standard Deviation; TNF, Tumor Necrosis Factor.

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and carotid intima media thickness (IMT) in a general population [9] and with increased risk of cardiovascular disease and mortality [10,11].

There is scarce information of OPG circulating levels in the MS, a cluster of cardiovascular risk factors.

The aims of the present work were 1) to evaluate OPG circulating levels in patients with the MS and its association with the presence of subclinical atherosclerosis and coronary arterial calcification and 2) to explore whether adipose tissue is a source of OPG.

Methods

Study population

This case control study was performed in 238 apparently healthy subjects (51% males, 60 ± 1 years; 49% women, 59 ± 1 years) attending the Cardiovascular Risk Area of the Clinic Universidad de Navarra for a general check-up. The demographic and clinical characteristics of the study population are summarized in Table 1.

All participants underwent a complete medical examination and anthropometric measurements were taken. Subjects were free from clinically apparent atherosclerotic disease based on the absence of history of coronary disease, stroke or peripheral artery disease, and normal electrocardiogram. Exclusion criteria were osteoporosis, impaired renal or liver function, cancer, and inflammatory diseases.

In order to exclude osteoporotic subjects, females under treatment with antiresorptive therapy were excluded from this study. Furthermore, in all females over 50 years old without treatment, the FRAX risk score was calculated. FRAX is an algorithm that determines fracture probability in individuals by integrating the weight of important clinical risk factors for fracture and mortality risk, with or without information on bone mass density. Women with FRAX scores higher than 3% (hip) or 20% (major) 10-year fracture risk were excluded from the study. Furthermore, women with confirmed osteoporosis by densitometry were excluded of the study.

Another group of patients ($n = 25$) who underwent elective surgery (for example abdominoplasty and laparoscopic gastric bypass) at the Surgery Department of the Clinic University of Navarra were recruited for the study in adipose tissue.

The MS was diagnosed according to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) guidelines. Individuals were classified as having the metabolic syndrome (MS) if they possessed three or more of the following criteria:

- High blood pressure: systolic and/or diastolic blood pressures were ≥130/85 mmHg or patients receiving blood pressure lowering drugs
- Hyperglycaemia: fasting plasma glucose ≥6.1 mmol/L (110 mg/dL) or patients receiving glucose lowering drugs
- Hypertriglyceridaemia: fasting plasma triglycerides ≥1.69 mmol/L (150 mg/dL)
- Low HDL-cholesterol: fasting HDL-cholesterol <1.04 or 1.29 mmol (40 or 50 mg/dL) in males and females, respectively
- Central obesity: waist circumference >88 or 102 cm in females and males, respectively.

Body mass index (BMI) was calculated using the following formula: weight (kg) / height^2 (m). Blood pressure was measured on the right arm, with the subjects in a seated position and after a 5 minute rest, with a mercury sphygmomanometer.

Waist circumference was measured at the superior border of the iliac crest.

Glomerular filtrating rate was estimated from plasma concentrations of endogenous creatinine employing the abbreviated four variable Modification of Diet in Renal Disease (MDRD) study equation (MDRD-4).

All participants signed an informed consent document, and the study was approved by the Local Ethics Committee for Human Research.

The percentage of patients without the MS taking statins was not significantly different compared with the number of patients with the MS on statins (23% vs 30%, non-significant).

Table 1
Demographic and clinical characteristics of the study population.

Variable	No MS	MS	*p*
Age, years	59 ± 1	64 ± 1	<0.01
Male, %	20.2	79.8	<0.001
Body mass index, kg/m^2	25.7 ± 0.25	29.8 ± 0.43	<0.001
Systolic arterial pressure, mm Hg	128 ± 1	142 ± 2	<0.001
Diastolic arterial pressure, mm Hg	76 ± 0.7	83 ± 1	<0.001
Waist circumference, cm	93 ± 0.8	102 ± 1	<0.001
Glucose, mg/dL	90 ± 1	111 ± 5	<0.001
Total cholesterol, mg/dL	212 ± 3	194 ± 6	<0.01
Triglycerides, mg/dL	88 ± 3	136 ± 7	<0.001
HDL-cholesterol, mg/dL	65 ± 1	47 ± 2	<0.001
LDL-cholesterol, mg/dL	129 ± 3	120 ± 5	n.s.

n.s., non-significant.

Carotid ultrasonography

All subjects underwent ultrasonography of the common carotid arteries performed with color duplex equipment (ATL 1500 HDI) coupled to a high-resolution linear transducer at a frequency of 5–12 MHz. The measurement of intima-media thickness (IMT) was performed in the far wall of the common carotid artery (10 mm proximal to the bifurcation). From each individual, the IMT was determined as the average of near- and far-wall measurements of each carotid artery. In addition, the presence or absence of atheromatous plaques was determined.

Examinations were carried out by a sonographer who was trained and experienced in performing sonographic examination and IMT measurements, and who was blinded to the participants' clinical information.

Computed tomography (CT) image acquisition protocol

A subgroup of 39 subjects underwent prospectively ECG-triggered cardiac computed tomography (CT) during a single breath hold using a DSCT system (Somatom Definition, Siemens Medical Solutions, Forchheim, Germany) at end inspiration. Acquisition started above the origin of the coronary arteries and ended at the dome of the diaphragm. The following protocol was used in all cases: tube voltage of 120 kV, current of 76 mAs for both tubes with online anthropomorphic tube current modulation (CareDose 4D, Siemens Healthcare), detector collimation of 6 × 3.0 mm, table displacement of 18 mm, and gantry rotation time of 0.33 ms (temporal resolution of 83 ms). ECG-gated cardiac CT studies were acquired at 70% or 40% of the cardiac cycle in individuals with heart rates <80 or ≥80 beats per minute, respectively, to obtain motion-free images of the coronary arteries. After manual adjustment of the field of view (FOV) to the heart, all data were reconstructed with 3 mm slice thickness, 1.5 mm reconstruction increment, and dedicated soft-tissue convolution kernel (B35f) in a range extending from the main pulmonary artery to the dome of diaphragm. No intravenous contrast media was administered.

Coronary artery calcium scoring

An experienced reader quantified the amount of coronary artery calcium (CAC) using dedicated software (CaScore, Siemens). Calcified lesions were identified as areas of at least 130 Hounsfield Units (HU) attenuation. The Agatston score was computed by multiplying the area of each lesion by a weighing factor that is dependent on the peak attenuation in the lesion. The scores of individual lesions were summed to obtain a global CAC Agatston score of all coronary vessels.

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