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Vaspin serum concentrations in patients with carotid stenosis

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ABSTRACT

Obesity is associated with accelerated atherosclerosis. Adipokines may directly influence vessel wall homeostasis by influencing the function of endothelial cells, arterial smooth muscle cells, and modulating inflammation. Recently, visceral adipose tissue-derived serpin (vaspin) was identified as a novel adipokine related to obesity and its metabolic consequences. However, the regulation of vaspin serum concentrations in human atherosclerosis is unknown. We therefore assessed vaspin serum concentrations in 107 consecutive patients with carotid stenosis undergoing carotid endarterectomy (CEA) in relation to severity of atherosclerosis, measures of obesity and circulating markers of obesity and atherosclerosis. Vaspin serum concentrations were significantly lower in patients with carotid stenosis who experienced an ischemic event within 3 months before surgery compared to asymptomatic patients. However, circulating vaspin was not associated with measures of atherosclerosis severity as maximum percentage stenosis. Vaspin serum concentrations were indistinguishable before and after CEA. We found a significant correlation between vaspin and leptin serum concentrations supporting previous results that vaspin closely reflects body fat mass.

In conclusion, our data show that low vaspin serum concentrations correlate with recently experienced ischemic events in patients with carotid stenosis despite the lack of an association between circulating vaspin and parameters of atherosclerosis severity.

1. Introduction

Obesity is associated with accelerated atherosclerosis. There are numerous mechanisms by which obesity can adversely affect the vasculature including changes in blood pressure, glucose level, lipid/lipoprotein metabolism, and systemic inflammation [1]. In addition, adipose tissue derived factors, so-called adipokines, may directly influence vessel wall homeostasis by influencing the function of endothelial cells, arterial smooth muscle cells, and macrophages in the vessel wall [1,2]. Adipokines have also been found within atherosclerotic plaques, suggesting local in addition to endocrine effects of these mediators in atherosclerotic lesions [3–5].

Visceral adipose tissue-derived serpin (vaspin) was recently identified as a member of serine protease inhibitor family, which was expressed in visceral adipose tissue of Otsuka Long-Evans Tokushima Fatty (OLETF) rats at the age when obesity and insulin plasma concentrations reach a peak [6]. Vaspin expression was shown to decrease with worsening of diabetes and body weight loss, whereas vaspin serum levels could be normalized by insulin or pioglitazone treatment [6]. We have recently demonstrated that human vaspin mRNA expression in adipose tissue of obese subjects is fat depot-specific [7] and that vaspin serum concentrations are elevated in obesity [8]. However, the regulation of circulating vaspin in human atherosclerosis has not been investigated. We therefore tested the hypothesis that elevated vaspin serum concentrations contribute to the relationship between obesity and premature and accelerated atherosclerosis.

In individuals with severe carotid stenosis, who have been submitted to our hospital for carotid endarterectomy (CEA), we sought to determine circulating vaspin in relation to measures of the extent of atherosclerosis, anthropometric and clinical characteristics as well as other circulating adipokines.

Abbreviations: CEA, Carotid endarterectomy; hs-CRP, high-sensitivity C-reactive protein; TIA, transient ischemic attack.

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2. Subjects and methods

2.1. Subjects

A total of 107 consecutive Caucasian men ($n = 73$) and women ($n = 34$) with extracranial carotid artery stenosis were included into the study. All patients, who have been submitted for carotid endarterectomy (CEA) to the Carotid Stenosis Group of the Department of Surgery, University of Leipzig have been recruited without any inclusion or exclusion criteria. The severity of carotid atherosclerosis in each subject was calculated by using maximum percentage of stenosis. The percentage of arterial stenosis and luminal narrowing was graded according to North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria by two experienced vascular radiologists in consensus using axial source images as well as curved planar reformations and digital subtraction angiography (DSA) as described [9,10].

Detailed clinical data were recorded for each patient (Table 1). Patients were classified into 2 groups, symptomatic ($n = 63$) or asymptomatic ($n = 34$), depending on whether or not the patients had experienced ipsilateral stroke, transient ischemic attack (TIA), or amaurosis fugax 6 months prior to surgery. Patients with asymptomatic carotid stenosis were detected incidentally during clinical examinations due to coronary artery disease (CAD), peripheral artery disease (PAD), or amaurosis fugax, TIA or stroke >6 months previously. Body mass index (BMI) of all subjects was calculated as weight divided by squared height. The patients were further subdivided into lean (BMI < 25 kg/m^2, $n = 33$), overweight (BMI 25–29.9 kg/m^2, $n = 52$), and obese patients (BMI ≥ 30 kg/m^2, $n = 22$). One day before CEA peripheral venous blood was collected between 8 and 10 a.m. after on overnight fast and was immediately centrifuged at $2500 \times g$ for 20 min to obtain serum. Samples were stored at −80 °C and thawed only once. Measurement of biochemical parameters was performed with standard procedures. The study was approved by the Ethics Committee of the University of Leipzig and the participating subjects signed an informed consent.

2.2. Assays

Vaspin was measured with a previously developed vaspin ELISA (AdipoGen, Inc.; Seoul, South Korea) (reference range of the assay: 0.016–1 ng/ml, sensitivity: 12 pg/ml) [8]. The reference range of normal vaspin values was 0.22–0.42 ng/ml. Leptin was measured with an ELISA (Mediagnost, Reutlingen, Germany; reference range of normal leptin values at a BMI of 25 kg/m^2: males: 1.2–8.9 ng/ml, females: 8–24 ng/ml). Plasma adiponectin was quantified with an ELISA (AdipoGen; Inc.; Seoul, South Korea; reference range of normal adiponectin values: 8.1–19.5 μg/ml). TNF-α was measured using an ELISA (R&D Systems, Inc., Minneapolis, USA; reference range of normal TNF-α values: <5 ng/ml). Serum C-reactive protein (CRP; immunoturbidometric latex test) and lipid analysis were performed with the ModularAnalytics EVO System (Roche Diagnostics GmbH, Mannheim, Germany). The leukocyte blood count was determined with the Sysmex XE-2100 analyzer (Sysmex GmbH, Bornbach, Germany).

2.3. Detection of vaspin mRNA and protein in the plaques

RNA was isolated from CEA samples containing advanced plaque with Trizol (Invitrogen GmbH, Karlsruhe, Germany) by standard protocol. cDNA was synthesized from 1 μg RNA in a 20 μl standard reaction mixture containing 200 U Superscript II RNaseH-reverse transcriptase (Invitrogen). Protein lysates were prepared in T-PER tissue protein extraction reagent (Perbio Science GmbH, Bonn, Germany) supplemented with protease inhibitor mixture Complete Mini (Roche). RT-PCR of vaspin mRNA and Western blotting using a human vaspin-specific monoclonal antibody (AdipoGen; Inc.; Seoul, South Korea) for vaspin were performed as previously described [7,8].

2.4. Statistical analyses

Data are shown as mean ± S.E.M. unless stated otherwise. Before statistical analysis, non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. The following statistical tests were used: paired Students's t test, Chi quadrate test and Pearson's simple correlation. Statistical relationship was performed using SPSS version 15.0 (Chicago, IL, USA). P values < 0.05 were considered to be statistically significant. Only correlations and group differences are considered to be significant based on a sufficient statistical power of ≥80%. Statistical power calculations revealed that our sample size ($n = 107$) is sufficient to detect significant correlations at a correlation coefficient >0.267 with a statistical power of 80%. Moreover, with a power of ≥80% we were able to detect significant differences of 0.27 ng/ml vaspin and of 0.8 ng/ml leptin between symptomatic and asymptomatic patients. The number of patients was sufficient to claim that vaspin levels discriminate between symptomatic and asymptomatic patients. Construction of receiver operating characteristic (ROC) curves confirmed that vaspin serum concentration significantly discriminates between groups of symptomatic and asymptomatic patients (AUC = 0.65, $p = 0.0059$).

3. Results

3.1. Vaspin serum concentrations in patients with carotid stenosis

Anthropometric and metabolic characteristics of 107 individuals with carotid stenosis are summarized in Table 1. Vaspin serum concentrations ranged from 0.07 to 3.28 ng/ml. There was no gender specific difference in serum vaspin levels (mean ± S.E.M.; females 0.63 ± 0.07 ng/ml, males 0.59 ± 0.07 ng/ml). We found highest vaspin serum concentrations in overweight (BMI 25.0–29.9 kg/m^2: 0.72 ± 0.09 ng/ml) as compared to both lean (BMI < 15.0 kg/m^2: 0.51 ± 0.06 ng/ml) and obese (BMI > 30 kg/m^2: 0.48 ± 0.13 ng/ml) individuals. There was a significant positive correlation between vaspin and leptin serum concentrations ($r = 0.35$, $p < 0.001$) and a significant negative correlation between circulating vaspin and CrP levels ($r = -0.28$, $p = 0.04$). In patients with carotid stenosis, vaspin serum concentration does not correlate with age ($r = 0.009$, $p = 0.9$), BMI ($r = 0.08$, $p = 0.4$), and type 2 diabetes (0.1, $p = 0.4$). Moreover, there was no correlation between circulating vaspin and the degree of carotid artery stenosis ($r = -0.04$, $p = 0.7$). On the other hand, we found a significant negative correlation between circulating vaspin levels and symptomatic carotid artery stenosis ($r = -0.3$, $p = 0.009$).

3.2. Serum vaspin was lower in symptomatic compared to asymptomatic patients

To further elucidate the significant relationship between circulating vaspin and symptomatic carotid stenosis, we divided our patients into two subgroups of either symptomatic or asymptomatic carotid stenosis patients (Table 1). Distinction between the groups was based on whether a patient had experienced ipsilateral stroke, transient ischemic attack (TIA), or amaurosis fugax 6 months prior to surgery. Patients with asymptomatic carotid stenosis were detected incidentally during clinical examinations due to coronary artery disease (CAD), peripheral artery disease (PAD), or amaurosis fugax, TIA or stroke in past medical history (>6 months from date of admission to the hospital). Serum vaspin concentrations