Atherosclerosis 232 (2014) 25–30

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

A polymorphism at *IGF1* locus is associated with carotid intima media thickness and endothelium-dependent vasodilatation

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ARTICLE INFO

Article history:
Received 5 September 2013
Received in revised form
4 October 2013
Accepted 17 October 2013
Available online 31 October 2013

Keywords:
IGF-1
Carotid intima media thickness
Atherosclerosis
Endothelial dysfunction

ABSTRACT

Objective: Whether IGF-1 has a protective or a detrimental role in vascular homeostasis remains unsettled. There is evidence that the C/T polymorphism rs35767 near the promoter region of the *IGF1* gene located in chromosome 12 is associated with plasma IGF-1 levels. We investigated the effects of this polymorphism on circulating IGF-1 levels, carotid intima media thickness (cIMT) and endothelial-dependent vasodilation.

Methods: Two samples of adult nondiabetic Whites were studied. Sample 1 comprised 1124 individuals in whom cIMT was measured by ultrasonography. Sample 2 included 162 drug-naïve hypertensive individuals in whom endothelium-dependent and endothelium-independent vasodilation were assessed by intra-arterial infusion of acetylcholine (ACh), and sodium nitroprusside (SNP), respectively. IGF-1 was determined by chemiluminescent immunoassay. rs35767 polymorphism was screened using a TaqMan allelic discrimination assay.

Results: In sample 1, IGF-1 levels were higher in subjects carrying the T allele compared with CC carriers (178 ± 78 vs. 166 ± 60 ng/mL, respectively; $P = 0.007$ adjusted for age, gender, and BMI). cIMT was lower in subjects carrying the T allele compared with CC carriers (0.71 ± 0.20 vs. 0.76 ± 0.22 mm, respectively; $P < 0.0001$ adjusted for age, gender, and BMI). In sample 2, maximally ACh-stimulated forearm blood flow was higher in subjects carrying the T allele compared with CC carriers (343 ± 191 vs. 281 ± 125%, respectively; $P = 0.02$ adjusted for age, gender, and BMI).

Conclusion: Subjects carrying the T allele exhibited significantly higher levels of circulating IGF-1, lower values of cIMT, and higher endothelium-dependent vasodilatation compared with CC carriers. These findings support the idea that IGF-1 plays a role in the pathogenesis of atherosclerosis.

Numerous experimental and clinical studies suggest that insulin-like growth factor-1 (IGF-1) system may be implicated in cardiovascular disease [1–6]. Infusion of IGF-1 into human brachial arteries increases blood flow through a nitric oxide (NO)-dependent mechanism [1,2], and, in human endothelial cells, IGF-1, interacting with its receptor, stimulates nitric oxide (NO) production [3]. Inhibitors of NO biosynthesis prevented IGF-1-induced vasodilatation in rats [4,5], and liver-specific IGF-1 knockout mice exhibited impaired acetylcholine-induced vasorelaxation of resistance vessels [6]. Lower plasma IGF-1 concentrations have been associated with many cardiovascular risk factors, including abnormal glucose homeostasis/diabetes [7,8] insulin resistance [9], lower HDL cholesterol [10], metabolic syndrome [11], metabolically abnormal obesity [12], endothelial dysfunction [13], left ventricular hypertrophy [14], inflammatory factors [15], and nonalcoholic fatty liver disease [16,17], all of which are potential contributors to the increased risk of cardiovascular disease associated with low plasma IGF-1 levels. Despite these and several other observations suggesting that low circulating IGF-1 levels within the normal range have a role in the development of cardiovascular diseases, prior studies examining the clinical outcomes of lower plasma IGF-1 concentrations have yielded mixed results. Cross-sectional studies using subclinical measures of atherosclerosis have reported an inverse relationship between circulating IGF-1 levels and carotid intima media thickness (cIMT) in healthy

Abbreviations: SNP, single nucleotide polymorphism; cIMT, carotid intima media thickness; EDV, endothelial-dependent vasodilation; FBF, forearm blood flow.

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.10.024

subjects [18] or atherosclerotic plaques in elderly subjects [19], and low circulating IGF-1 concentrations have been associated with angiographically documented coronary artery disease [20]. Moreover, in individuals with acute myocardial infarction, serum IGF-1 levels on admission to hospital were reduced compared with matched controls, and were associated with subsequent adverse events including death, recurrent ischemia, revascularization and sustained ventricular tachycardia [21]. Several prospective studies have suggested that low circulating levels of IGF-1 are associated with increased risk of ischemic heart disease [22], nonfatal myocardial infarction [23], ischemic heart disease mortality [24], and cardiovascular mortality [26].

In contrast with these findings, other studies have reported that high circulating IGF-1 concentrations are independently associated with angiographically assessed coronary heart disease [27], common carotid artery IMT [28], and coronary artery disease progression in young male survivors of myocardial infarction [29]. In addition, in a cross-sectional study carried out in 6773 primary care patients, circulating IGF-1 concentrations were reported to be associated with increased risk for coronary artery disease [30]. Thus, whether IGF-1 has a protective or a detrimental role in vascular homeostasis remains a subject of debate.

In an attempt to clarify this issue, we took advantage of the opportunity to study the association of an IGF-1-raising polymorphism (rs35767) near the promoter region of the *IGF1* gene located in human chromosome 12 with early signs of atherosclerosis, and with endothelial-dependent vasodilation. Previous studies have repeatedly reported that carriers of the T allele of polymorphism rs35767 have increased levels of circulating IGF-1 as compared with CC carriers [31–34]. In the present study, we investigated the effects of the rs35767 polymorphism on circulating IGF-1 levels, carotid artery IMT, and endothelial-dependent vasodilation in two cohorts of nondiabetic White Europeans.

1. Methods

Study subjects. Two different samples of adult (≥20 years of age) nondiabetic individuals of European ancestry were studied.

Two cohorts were enrolled: sample 1 comprised 1124 nondiabetic individuals, and sample 2 included 162 drug-naïve hypertensive individuals. cIMT was measured by ultrasonography in sample 1.

Sample 1 comprised 1124 nondiabetic individuals consecutively recruited at the Department of Systems Medicine of the University of Rome-Tor Vergata and at the Department of Medical and Surgical Sciences of the University "Magna Graecia" of Catanzaro [35]. The inclusion criteria were: fasting plasma glucose <126 mg/dL and presence of one or more cardio-metabolic risk factors including, hypertension, dyslipidemia, and overweight/obesity. Exclusion criteria comprised: history of any malignant disease, end stage renal disease, chronic gastrointestinal diseases, chronic pancreatitis, positivity for antibodies to hepatitis C virus (HCV) or hepatitis B surface antigen (HBsAg), and use of medications able to modify glucose metabolism including corticosteroids, glucose-lowering, lipid-lowering and antihypertensive therapies. After 12-h overnight fasting, subjects underwent anthropometrical evaluation and a venous blood sample was drawn for laboratory determinations. Total serum IGF-1 concentrations were determined by chemiluminescent immunoassay (Nichols Institute Diagnostic, San Juan Capistrano, CA).

High resolution B-mode ultrasound was used to measure IMT of the common carotid artery by using an ATL HDI 3000 ultrasound system (Advanced Technology Laboratories, Bothell, WA) equipped with a 7.5 MHz transducer, as previously described [36]. Manual measurements were conducted in plaque-free portions of the 10-mm linear segment proximal to the carotid bulb. Plaque was defined as a clearly isolated focal thickening of the intima-media layer with a thickness >1.3 mm. For each subject two measurements were performed bilaterally, and the values were averaged. Ultrasound examination was performed by a single skilled examiner who was unaware of the subjects' clinical and laboratory findings. The intra-observer variability of IMT measurements was tested in 120 randomly chosen scans; the correlations between the two readings was $r = 0.95$, and the absolute mean difference was 4.6 ± 2.6% A value of cIMT >0.9 mm was used as index of vascular abnormality according to the 2013 Guidelines for the management of arterial hypertension released by the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) [37].

In order to get further insights on the role of the rs35767 polymorphism on vascular function, an additional sample of nondiabetic individuals was analyzed. Sample 2 included 162 drug-naïve hypertensive individuals consecutively recruited at the Department of Medical and Surgical Sciences of the University "Magna Graecia" of Catanzaro as previously described [38]. None of the subjects had a history or clinical evidence of angina, myocardial infarction, valvular heart disease, diabetes mellitus, hypercholesterolemia, peripheral vascular disease, or coagulopathy. Forearm blood flow (FBF) measurements were performed by strain-gauge plethysmography accordingly to a previously described method [39,40]. FBF and blood pressure were assessed during intra-arterial infusion of saline, acetylcholine (ACh), and sodium nitroprusside (SNP). Endothelium-dependent and -independent vasodilations were measured by a dose–response curve to intra-arterial ACh (7.5, 15, and 30 μg mL^{-1} min^{-1}, each for 5 min) and SNP infusions (0.8, 1.6, and 3.2 μg mL^{-1} min^{-1}, each for 5 min), respectively. Each patient's FBF maximal response to ACh or SNP was considered for statistical analysis. Forearm vascular resistance (VR), expressed in arbitrary units (U), was calculated by dividing mean blood pressure at each dose point by FBF. Inter- and intra-observer variability was 3.3 and 2.7%, respectively.

The studies were approved by Institutional Ethics Committees and written informed consent was obtained from each subject in accordance with principles of the Declaration of Helsinki.

1.1. DNA analysis

DNA was isolated from whole blood using commercial DNA isolation kit (Promega, Madison, WI). Screening of rs35767 polymorphism was performed using a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). TaqMan genotyping reaction was amplified on a GeneAmp PCR system 2700 and fluorescence was detected using an ABI Prism 7000 sequence detector (Applied Biosystems, Foster City, CA). Genotyping quality was tested by including 3 HapMap samples in each 96-well plate. The agreement rate with the HapMap database genotypes was >99%.

1.2. Statistical analysis

Due to a skewed distribution, triglycerides values were logarithmically transformed before the statistical analyses. The results for continuous variables are given as means ± SD. Categorical variables were compared by χ^2-test. Differences of continuous variables between groups were tested after adjusting for confounding factors such as age, gender, adiposity, blood pressure, fasting glucose, and lipid levels by ANCOVA (general linear model). The Hardy–Weinberg equilibrium between the genotypes was evaluated by χ^2 test. Genotype distributions were in Hardy–Weinberg equilibrium ($P > 0.05$). Power calculations were performed with