Presence of irregularity in region between −1115 and −784 nt in P1 promoter of Insulin-Like Growth Factor-1 gene may indicate beneficial effect on coronary arteries in a group of patients with stable angina: preliminary data

Abstract Insulin-like growth factor-1 (IGF-1) plays an important role in arterial homeostasis. Its properties seem to depend on circulating IGF-1 level changes. The various IGF-1 levels are caused by varied expression of IGF-1 gene, due to the polymorphic structure of IGF-1 gene or its regulatory sequences. We examined the P1 promoter, being responsible for most IGF-1 transcripts, in patients with stable angina, to evaluate its sequence changes and to assess its influence on protein synthesis as well as on the degree of arteriosclerosis. For that purpose we evaluated the DNA isolated from blood cells. The DNA was amplified by using polymerase chain reaction (PCR), then analyzed using the SSCP (single-strand conformation polymorphism) technique. Products of every stage were verified by electrophoresis on agarose gel. In addition, every patient had coronary angiography performed and IGF-1, IGFBP3, and lipid levels measured. The SSCP in the region between −1115 and −784 nt was less commonly observed among subjects with positive MI (myocardial infarction) familial history (P = 0.0008) and with MI history (P = 0.012) than in patients without these conditions. Subjects with this irregularity tended towards higher circulating IGF-1 levels. In addition high Gensini scores – over 95th percentile, 105 points in our study – were more frequent in SSCP patients (P = 0.03). We presume that presence of SSCP in the P1 region between −1115 and −784 nt may positively affect coronary arteries by increasing circulating IGF-1 levels, but its clinical importance requires molecular verification and further studies.

Key words Insulin-like growth factor-1 · Growth hormone · Coronary artery disease · IGF-1 gene · P1 promoter polymorphism

Introduction

Insulin-like growth factor-1 (IGF-1) is considered to be involved in the regulation of vessel homeostasis, glucose and lipid metabolism.1-3 Disturbances in that process may lead to vascular pathologies including arteriosclerosis.3 Protein synthesis is controlled and regulated by various mechanisms and enzymes. Each of these mechanisms may contribute to changes in phenotype protein levels.4-5 It is also suggested that the efficacy of that process depends on gene structure or gene regulatory sequences.

The potential role of increased or decreased IGF-1 and other cytokines serum/plasma levels in the development of coronary arteriosclerosis, has been extensively studied.4-6 In addition, several data have defined the impact of particular molecular variants of IGF-1 gene and its P1 promoter on IGF-1 serum/plasma level changes. P1 is considered to be responsible for most IGF-1 gene transcripts. One known studied variation of P1 promoter is the 192nt allele (RS 10665874), having a different number of dinucleotide CA copies.7 Its frequency in population of subjects of up to 55 years was described in the epidemiological Rotterdam Study.7,8 No data has clarified the relation of this or other polymorphisms to the circulating level of IGF-1 as well as the effect of polymorphisms and/or IGF level modification on the development of coronary arterial disease (CAD). In addition, the association of various polymorphisms in the
P1 region between −1115 and −784 nt with biochemical parameters in patients with stable angina undergoing coronary angiography has not been studied as yet. Furthermore, its relationship to lipid or glucose metabolism is still poorly documented and remains unclear.

The purpose of this study was to analyze the P1 promoter (region between −1115 and −784 nt) of IGF-1 gene, and examine the relationship of its potential irregularity with circulating IGF level changes. In addition, we intended to assess the common relation of promoter irregularity and circulating IGF-1 level changes with the development of coronary arteriosclerosis in patients undergoing routine coronary angiography. Our purpose was also to correlate the promoter irregularity with medical history data.

**Patients and methods**

Blood samples for plasma isolation and for isolation of genomic DNA with salt-extraction approach were collected from 101 consecutive patients undergoing routine coronary angiography. Using the polymerase chain reaction (PCR), analyses of sequences of the promoter P1 of IGF-1 gene between −1115 and −784 nt were performed on each sample. The products were verified by electrophoresis on agarose gel and analyzed using single-strand conformation polymorphism (SSCP) to evaluate IGF-1 promoter gene polymorphism. DNA fragments were separated by electrophoresis and subsequently underwent silver staining. A Beckman–Coulter Genetic Analysis System CEQ 2000XL was used for automated sequencing, allowing more accurate determination of nucleotide sequence changes essential for the identification of gene promoter structure. The SSCP images differing from controls indicated the irregularity in DNA sequence. IGF-1 serum levels were measured by radioimmunoassay technique with the use of commercial kits from Biosource (Nivelles, Belgium). The lipid levels were measured using enzymatic kits of Architect system (Abbott, San Diego, CA, USA). The coronaryography images were analyzed in quantitative coronary angiography (QCA) and the Gensini score was calculated for quantification of arteriosclerotic changes. In addition, body mass index (BMI) and IGF-1/BMI ratio (for better evaluation of IGF level per kilogram and m²) were calculated. All known clinical conditions which may lead to increased or decreased IGF-1 levels, i.e., acute coronary syndrome, acute circulatory insufficiency (New York Heart Association grade III/IV), acute or chronic insufficiency of bone marrow, neoplastic disease, rheumatic disease, or other inflammatory disease were the exclusion criteria.

The Shapiro–Wilk W-test was employed in the assessment of normality. At normal distribution of variables we used the Student t-test (for two independent variables) or Levene test and analysis of variances (for multiple independent samples). The Mann–Whitney test (for two independent variables) and Kruskal–Wallis (for multiple independent samples) test were used at abnormal distribution of variables. We also utilized cross-tabulation tables with one- and two-tailed Fisher exact and χ² Pearson tests. Statistical analysis was computed using STATISTICA 7.0 software (StatSoft, Tulsa, OK, USA). The results are given as mean value ± SD.

Patients’ history was collated on the basis of hospital discharge letters and patients’ medical records. The protocol of the study was accepted by the local committee on human research. Every patient was individually informed and received a study consent form to be signed.

**Results**

Considerable arteriosclerotic lesions (above 50% of the vessel lumen) in one coronary artery were observed in 22 subjects (17 male and 5 female subjects), in two arteries in 15 patients (11 male, 4 female), whereas three-vessel disease was diagnosed in 22 patients (12 male, 10 female). No significant changes (less than 30%) in coronary arteries were found in 32 patients and a group of 10 patients had arteriosclerotic changes between 30% and 50% of artery lumen. Thirty-eight patients (24 male, 14 female) had a history of myocardial infarction (MI), 36 patients (20 male, 16 female) had a family history of coronary arterial disease (CAD), and history of stroke was positive in 5 patients. Basic clinical parameters are shown in Table 1.

Single-strand conformation polymorphism in P1 promoter of IGF-1 gene in the region between −1115 and −784 nt was found in 33 subjects. Polymorphism RS 11829693 in rare genotype A/A was confirmed in two of these cases. The circulating IGF-1 levels tended towards higher values in subjects with this genotype (230.2 ± 59.2 ng/ml vs 189.6 ± 46.9 ng/ml), but the small number of individuals negated statistical analysis. The characteristics of the studied group are shown in Tables 2 and 3. The SSCP was more frequently observed in advanced coronary arteriosclerosis according to Gensini score (above 95th percentile vs Gensini score = 0, respectively P = 0.03) (Fig. 1), similar to diabetic patients (P = 0.04). Additionally, SSCP was in evidence more frequently in arteriosclerotic cases, related to three-vessel disease (P = 0.06). The MI history (6.25%) in patients (aged between 46–74) as well as positive familial CAD history (5.68%) were significantly more seldom present in individuals with irregularity in P1 promoter of IGF-1 gene in comparison to those without SSCP (31.25%, P = 0.012 for MI history, 35.23% P = 0.00088 for positive CAD familial history, respectively) (Fig. 2). There was no significant relationship between the presence of irregularity in P1 promoter and low-density lipoprotein (LDL) high-density lipoprotein, total cholesterol, and triglyceride levels.

**Discussion**

Insulin-like growth factor-1 is a growth factor with multiple biological properties, which are related to the process of attaching serine kinase membrane receptor linked to insulin