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Circulating interleukin-18: Association with IL-8, IL-10 and VEGF serum levels in patients with and without heart rhythm disorders

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ABSTRACT

Background: An altered IL-18 pathway in patients with coronary artery disease has recently been described and this cytokine was shown to be of clinical and prognostic utility. Cardiomyocytes are a target of this cytokine which exerts inflammatory, hypertrophic and profibrotic activities.
Objectives: The aim of the study was to verify the ability of IL-18 to induce expression of other pro/anti-inflammatory cytokines and to analyse the relationship between these molecules in serum from patients with heart rhythm disorders and coronary artery disease.
Methods: All patients in the study were divided into two groups: with heart rhythm disorders and without but with diagnosed coronary artery disease. Heart rhythm disorders included sinus node dysfunction, bradycardia and tachycardia.
Results: The interrelationships among all tested clinical, biochemical and inflammatory parameters with a dependence on median IL-18 values were checked. From all tested parameters only cytokines IL-8, IL-10 and VEGF, were associated with the serum IL-18 levels.
Conclusion: IL-18, IL-8 and VEGF were identified as a factors involved in heart rhythm disorders and coronary artery disease patho-physiology and regarded as markers of prognostic significance and potential therapeutic targets. The demonstration of their in vivo relationship added new insights into the understanding of the interdependence of inflammatory pathways in patients with heart rhythm disorders or coronary artery disease.

1. Introduction

Interleukin-18 (IL-18, formerly IFN-γ-inducing factor) is a newly-described cytokine that has been shown to be essential for IFN-γ production [1]. Although IL-18 by itself, induces almost no IFN-γ, IL-18 greatly potentiates IFN-γ production in the presence of IL-12 [2]. IL-18 and IL-12 act synergistically to induce the production of IFN-γ in T cells, natural killer cells, and subsets of macrophages [3–5]. Recent studies have reported that in addition to its costimulatory activities in the production of IFN-γ; IL-18, also has direct pro-inflammatory effects, able to activate natural factor kappa B (NF-κB) [6], and to induce production of pro-inflammatory cytokines and chemokines such as a tumour necrosis factor-α (TNF-α), IL-1β, IL-6 and IL-8 [7,8]. This capacity to induce pro-inflammatory cytokines seems to place IL-18 in a commanding role in the coordination of immune responses [2]. IL-18 is known to display inflammatory, hypertrophic, and pro-apoptotic properties and acts through the interaction with a complex receptor, including a binding chain (IL-18Rα) and a signalling chain (IL-18Rβ) [9,10].

Recent experimental studies [11,12], have proven that the administration of exogenous IL-18 caused myocardial dysfunction, probably due to the expression of inducible nitric oxide synthase and myocardial depressant nitric oxide production. Another important role of IL-18 was reported in atherosclerotic plaque progression and destabilization [11–15]. These results were highlighted by the observation that serum levels of IL-18 were strong predictors of cardiac mortality in patients with coronary artery disease (CAD) [16].

All the above studies, reported an association between increased circulatory IL-18 concentration and different heart pathologies. The aim of the present study was to investigate whether there were any associations between the circulatory IL-18 levels and standard clinical and biochemical markers and cytokines in patients with heart rhythm disorders (HRD) and patients without HRD but with (CAD). In addition,

Abbreviations: LVEF, left ventricular ejection fraction; LAV, left atrium volume; RAV, right atrium volume; PASP, pulmonary artery systolic pressure; PAD, pulmonary artery diameter.

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Puren et al. [7], have recently shown that the pro-inflammatory cascade induced by IL-18 in human mono-nuclear cells is driven by a TNF-α-like molecule. We have been focused on the associations between proinflammatory cytokines and IL-18. In order to check its modulatory impact on the cytokine inducing pathways we employed some anti-inflammatory cytokines.

2. Materials and methods

2.1. Patient data

The study group consisted of 74 patients with HRD (38) and without HRD but with diagnosed CAD (36). HRD was confirmed by 24 h Holter echo-cardiographic monitoring, instituted the same day after an echo-cardiogram had been evaluated and blood samples taken for biochemical analysis. HRD included sinus node dysfunction (SND) (22), tachycardia (9) and bradycardia (7). The study group consisted of 28 female and 46 male patients.

All patients were stable with regards to symptoms and therapy for at least one month prior to the measurements conducted within this study. Exclusion criteria were: active infection, allergy, asthma, diabetes of any type, cancer, inflammatory diseases, patients with sleep apnea, respiratory insufficiency of any genesis and treatment with anti-inflammatory drugs.

The patients were on standard medication consisting of ACE inhibitor (34.66%), anti-arrhythmic agents (17.33%), anticoagulants (38.66%), antiplatelet drug (42.66%), β-blockers (57.3%), calcium channel blockers (34.66%), diuretics (17.3%), hypolipidemic agents (68%) and proton-pump inhibitors (25.3%). History of systolic blood pressure (34.67%), systemic hypertension (64%) and chronic renal failure (1.33%) were also assessed (Table 1).

Patients were treated according to the guidelines of the American Heart Association/American College of Cardiology. The study conformed to the principles outlined in the Helsinki Declaration and was approved by the Institutional Review Board (Ethics Committee of the Russian National Research Medical University N.I. Pirogov). After receiving appropriate information all recruited participants gave informed consent for the study.

2.2. Blood collection and biochemical analysis

Blood samples were obtained when patients were first admitted from the ulnar vein between 8 and 9 a.m. after an overnight fast. Serum was prepared within 1 h by centrifugation at 2000 g at room temperature for 15 min. The serum was stored at −80 °C until analysis were performed. Serum samples were analysed for: triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), sodium (Na), glucose (Glu), creatinine (CRT) and urea (U) by an Automatic biochemical Analyser (Abbott Architect c8000 Chemistry Analyser).

Table 1
Study patient characteristics.

Medications	
β-Blockers (n, %)	43 (57.3%)
Hypolipidemic agents (n, %)	41 (54.7%)
AT_1-receptor antagonists (n, %)	10 (13.3%)
Diuretics (n, %)	13 (17.3%)
Proton-pump inhibitor (n, %)	19 (25.3%)
ACE inhibitor (n, %)	26 (34.7%)
Antiplatelet drug (n, %)	32 (42.7%)
Antiarrhythmic agents (n, %)	13 (17.3%)
Anticoagulants (n, %)	29 (38.7%)
Calcium channel blockers (n, %)	26 (34.7%)
Diseases	
Systemic hypertension (n, %)	48 (64%)
Chronic renal failure (n, %)	1 (1.33%)
Systolic blood pressure (mm Hg ± SD)	135.96 ± 19.07
IL-18 (pg/ml)	122.24 ± 66.47

2.3. Immuno-assays

IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-18 and VEGF in the serum were analysed by the newly developed ELISA for quantitative analysis of IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-17, IL-18, and VEGF levels from Bender Med-Systems. The limits of detection of the assays were about 1.0 pg ml^{-1} for IL-1β, 2.0 pg ml^{-1} for IL-2, 0.4 pg ml^{-1} for IL-4, 0.92 pg ml^{-1} for IL-6, 2.0 pg ml^{-1} for IL-8, 1.0 pg ml^{-1} for IL-10, 2.0 pg ml^{-1} for IL-17, 2.0 pg ml^{-1} for IL-18 and 10.0 pg ml^{-1} for VEGF. Inter- and intra-assay CVs were 6.4% and 3.1% for IL-1β, 7.7% and 5.8% for IL-2, 8.3% and 8.6% for IL-4, 5.2% and 3.4% for IL-6, 8.9% and 6.1% for IL-8, 9.8% and 9.4% for IL-10, 4.8% and 8.5% for IL-17, 5.8% and 7.6% for IL-18, and 8.1% and 9.7% for VEGF.

2.4. Diagnostic methods

Electrocardiograms (ECG) from the patients were recorded before blood sampling. The following time domain variables were computed for each subject: heart rate (HR), PQ interval, QRS complex, QT interval. Echo-cardiograms (Echo-CG) were also recorded before blood sampling, measuring the following parameters: beats per minute (BPM), left ventricular ejection fraction (LVEF), left atrial volume (LAV), left ventricular stroke volume (LVSV), left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), pulmonary artery systolic pressure (PASP) and right atrial volume (RAV).

2.5. Statistical analysis

Data was summarized and displayed as means ± SD for continuous variables, inclusive of the number of patients plus percentages in each group for categorical variables. The distribution was tested by the one-sample Kolmogorov–Smirnov test, while the Mann Whitney U analysis was used for non-normally distributed continuous variables. For categorical variables, chi-square statistics were used for assessing overall significance between the two groups of patients comparing the high and low plasma levels of IL-18. Correlations between the markers were calculated using Pearson correlation analysis. The level of significance for analysis was two-tailed and $p < 0.05$ was considered statistically significant. All analyses were performed with Graph Pad Prism 5.0 (San Diego, CA, USA).

3. Results

3.1. Differences between groups with and without HRD

The primary goal of this study was to check the differences between IL-18 serum levels, ECG, and Echo-CG parameters in patients with HRD versus patients without HRD but with CAD. The IL-18 levels of these two groups were compared and there were no significant differences between patients with or without HRD ($p = 0.51$). Echo-CG and ECG parameters were also insignificant in both tested groups ($p = 0.35$, 0.81, 0.26, 0.84, 0.79, 0.71, 0.36, 0.77, 0.52, 0.73 and 0.62, for HR, PQ, QRS, QT, LVEF, LAV, PASP, LVSV, LVEDV, LVESV, and RAV; respectively, Table 2).

Further, in order to check the influence of gender on the circulatory IL-18 levels within both groups (with and without) HRD, we compared: male with ($n = 20$) versus female with ($n = 18$) HRD, ($p = 0.06$) and male without ($n = 26$) versus female without ($n = 10$) HRD ($p = 0.23$). The changes in circulatory IL-18 levels within the same gender was followed by comparing male with ($n = 20$) versus male without ($n = 26$) HRD ($p = 0.96$) and female with ($n = 18$) versus female without ($n = 10$) HRD ($p = 0.62$). The values of IL-18 were different within