Clinica Chimica Acta 461 (2016) 19–24

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

Increased IL-37 concentrations in patients with arterial calcification

Kunwu Yu [a,1], Xiaohong Min [b,1], Yingzhong Lin [c], Ying Huang [d], Song Huang [e], Ling Liu [c], Yudong Peng [a], Kai Meng [a], Dazhu Li [a], Qingwei Ji [c,*], Qiutang Zeng [a,*]

[a] Laboratory of Cardiovascular Immunology, Institute of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China
[b] Department of Pathology, Puren Hospital, Wuhan University of Science and Technology, Wuhan, China
[c] Department of Cardiology, the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China
[d] Department of Ultrasound, the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, China
[e] Department of Orthopedics, Liyuan Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 14 June 2016
Accepted 18 July 2016
Available online 20 July 2016

Keywords:
IL-37
Arterial calcification
Inflammation

ABSTRACT

Background: Our previous study indicates that IL-37 plays a critical role in both atherosclerosis and arterial calcification. However, whether IL-37 concentrations are significantly changed in patients with arterial calcification has not yet been investigated.
Methods: Anterior tibial arterial wall specimens were obtained from 8 patients with type 2 diabetes mellitus and 8 patients who experienced a traffic accident. IL-37 expression was measured by immunohistochemistry in the calcified and the normal samples. In addition, plasma IL-37 concentrations were measured in *75* patients with coronary artery calcification (CAC) and *50* patients without coronary artery calcification (NCAC).
Results: High concentrations of IL-37 were detected in calcified samples, *whereas* low concentrations of IL-37 were detected in the normal arteries. Macrophages and vascular smooth muscle cells were the main source of IL-37. *P*lasma IL-37 concentrations were *significantly* increased in CAC patients compared with NCAC patients. A correlation analysis showed that IL-37 *was* positively correlated with age, fasting glucose, alkaline phosphatase, IL-6, TNF-α, C-reactive protein and Agatston scores. Binary logistic regression analyses demonstrated that fasting glucose and IL-37 were independently associated with the presence of CAC.
Conclusions: Increased IL-37 concentrations are associated with the onset of arterial calcification.

1. Background

Arterial calcification is a common vascular pathology associated with atherosclerosis, hypertension, diabetes and chronic kidney disease [1–3]. Arterial calcification has 2 main types, atherosclerotic calcification, which mainly results from hyperlipidemia and hypertension, and medial calcification which is mainly induced by diabetes and chronic kidney disease. Atherosclerotic calcification and medial calcification occur frequently in concert and contribute synergistically to atherosclerotic disease, although they have different causes. Multiple clinical studies have demonstrated that arterial calcification is a predictor of future cardiovascular disease events beyond traditional risk factors [4–7]. Although the process of arterial calcification appears to be a regulated process similar to bone formation, the underlying mechanisms that drive and regulate the process of arterial calcification remain unknown. Accumulating evidence has revealed that inflammation plays a critical role in the development of atherosclerosis and arterial calcification and also serves as a bridge between atherosclerosis and arterial calcification [3,8,9]. Data from animal experiments have confirmed that proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, promote the differentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells and exacerbate the arterial calcification process [10–14]. Clinical studies have found that circulating inflammatory mediators, such as C-reactive protein (CRP) and TNF-α, are independently associated with an increased incidence of arterial calcification [15–21].

IL-37, formerly known as IL-1F7, is a novel anti-inflammatory cytokine in the IL-1 ligand family that consists of 11 members [22]. IL-37 has five splice variants (IL-37a-e), and IL-37b is the main isoform that exists in peripheral blood. IL-37 can decrease the production of proinflammatory cytokines and protect mice from inflammatory and autoimmune diseases [22–24]. Previously, we determined that IL-37 concentrations are significantly increased in patients with coronary artery disease and that recombinant IL-37 ameliorates atherosclerosis and myocardial ischemia in mice, indicating a close relationship between IL-37 and cardiovascular disease [25–27]. In addition, we demonstrated

Abbreviations: CAC, coronary artery calcification; GLU, fasting glucose; Hcy, homocysteine; IL, interleukin; VSMC, vascular smooth muscle cell.

* Corresponding authors.
E-mail address: jqw124@163.com (Q. Ji).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cca.2016.07.011
0009-8981/

that recombinant human IL-37 attenuates arterial calcification in ApoE$^{-/-}$ mice by promoting the production of osteoprotegerin, suggesting that IL-37 plays a protective role in arterial calcification [27]. However, it is unknown whether endogenous IL-37 is associated with the onset of arterial calcification in humans.

2. Methods

2.1. Human tissue sample preparation and immunohistochemistry

We investigated whether IL-37 expression was increased in calcified human arteries. Anterior tibial arterial wall specimens containing calcified lesions were obtained from 8 patients with type 2 diabetes mellitus (T2DM) (6 men, 2 women; age, mean ± SD: 67 ± 11 y; range: 50 to 80 y) who underwent amputations in the Department of Orthopedics, Liyuan Hospital. The clinical characteristics of the patients are listed in Table 1. Anterior tibial arterial specimens free of calcification were obtained from 8 patients who had been injured in a traffic accident. After surgery, the samples were fixed in formalin and embedded in paraffin for histology. Tissues sections were routinely stained with hematoxylin-eosin or anti-human IL-37 antibody. To determine the cellular localization of IL-37, an anti-CD3 antibody, an anti-CD68 antibody and an anti-α-SMA antibody were used to identify T cells, macrophages and VSMCs in calcified arterial specimens. For the quantitative immunohistochemistry analysis, images were visualized and analyzed using the HMIAS Series Color Medical Image Analysis System (Champion Image Ltd.). Written informed consent was obtained from each patient. The study was approved by the Ethics Committee of Union Hospital and the People's Hospital of Guangxi Zhuang Autonomous Region. Written informed consent was obtained from each patient.

2.2. Coronary artery calcification patients

We recruited 125 patients who underwent helical computed tomography examinations using the Siemens Somatom Definition Scanner (Siemens) or Toshiba Aquillion One 320 Slice CT Scanner (Toshiba) between September 2012 and April 2013 at Union Hospital and the People's Hospital of Guangxi Zhuang Autonomous Region, China. Coronary artery calcification (CAC) was measured with a standardized protocol by trained readers using dedicated hardware and software. An Agatston score was computed by summing the calcium score for each of the epicardial arteries (left main, left anterior descending, diagonals, circumflex, and right coronary). CAC was considered present if the Agatston score was ≥1, whereas an Agatston score of 0 was considered to indicate an absence of CAC (NCAC). Therefore, the patients were classified into 2 groups: the CAC group (41 men and 34 women, mean age of 61.6 ± 8.9 y) and the NCAC group (25 men and 25 women, mean age of 57.3 ± 8.7 y). For additional analyses, CAC patients were divided into 2 groups: the low-grade CAC (LCAC) group (23 men and 21 women, mean age of 60.4 ± 9.6 y) with an Agatston score from 1 to 399 and the remarkable CAC (RCAC) group (18 men and 13 women, mean age of 63.3 ± 7.7 y) with an Agatston score ≥400.

Patients with clinical cardiovascular disease, thromboembolism, collagen disease, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, septicemia, or other inflammatory disease, as well as those who were on steroid therapy, were excluded from the study.

Table 1
Clinical characteristics of patients.

Characteristics	Control	Diabetes	*P* value
Age (y)	37 ± 12	67 ± 11	0.00
Sex (male/female)	5/3	6/2	NS
Hypertension, n (%)	–	6(75)	
CHD, n (%)	–	1(12.5)	
Smoking, n (%)	2 (25)	2(25)	NS
BMI(Kg/m^2)	25.7 ± 3.8	28.4 ± 3.1	NS
SBP(mm Hg)	113 ± 15	133 ± 24	0.09
DBP(mm Hg)	73 ± 8	82 ± 17	NS
GLU(mmol/l)	4.93 ± 0.46	9.15 ± 2.70	0.00
HbA1C (%)	4.82 ± 0.46	7.31 ± 1.24	0.00
TG (mmol/l)	1.52 ± 0.79	2.00 ± 2.48	NS
TC (mmol/l)	3.90 ± 0.95	3.95 ± 0.94	NS
HDL-C (mmol/l)	1.11 ± 0.17	1.01 ± 0.25	NS
LDL-C (mmol/l)	2.16 ± 0.68	2.27 ± 0.90	NS
Creatinine (μmol/l)	63.02 ± 12.86	75.73 ± 15.91	NS
CRP (mg/l)	1.45 ± 1.01	2.45 ± 1.67	NS

The data are given as the mean ± SD. or number of patients. SBP: systolic blood pressure; DBP: diastolic blood pressure; OAD: oral antidiabetic drugs.

2.3. Laboratory measurement

Fasting blood samples were obtained the morning following admission for these patients. The samples were collected into sodium heparin Vacutainers (Becton-Dickinson). The blood was centrifuged for 10 min at 2000 ×*g* and the plasma was stored at −80 °C until further use.

The concentrations of plasma IL-37 (Adipogen AG), IL-6 and TNF-α (Neobioscience) were measured by an enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions. The minimal detectable concentration was 10 pg/ml for IL-37. The ELISA intra-assay and inter-assay coefficients of variation were <5% and <10%, respectively. The minimal detectable concentrations were 3 pg/ml for IL-6 and 7 pg/ml for TNF-α. The ELISA intra-assay and inter-assay CVs were <9% for IL-6 and <9.5% for TNF-α, respectively. All samples were measured in duplicate.

The concentrations of lipid and lipoprotein fractions, fasting glucose, HbA1C, alkaline phosphatase (ALP), creatinine, uric acid *(UA)*, CRP, homocysteine *(Hcy)*, calcium and phosphate at baseline were measured in the central laboratory of Union Hospital.

2.4. Statistical analysis

All of the data are presented as the mean ± SD. When comparing only 2 groups, Student's *t*-test was used. For comparisons involving 3 groups, one-way ANOVA followed by Neuman-Keuls post-hoc test was used. Spearman's correlation was used to calculate the correlations between plasma IL-37 concentrations and the other parameters. To identify the independent predictors of the presence of CAC, simple linear regression analyses were performed with the following candidate variables entered into the model: age, sex, hypertension, diabetes, smoking, body mass index (BMI), lipid and lipoprotein fractions, fasting glucose, HbA1C, ALP, creatinine, *UA*, CRP, *Hcy*, calcium, phosphate, IL-37, IL-6 and TNF-α. Next, the variables that exhibited a trend ($P < 0.05$) toward an association with the presence of CAC were included in subsequent binary logistic regression analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. In all of the tests, a value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. IL-37 expression in human calcified arteries

The baseline characteristics of the patients are presented in Table 1. Fig. 1A shows a normal artery, whereas Fig. 1C shows a calcified artery. As displayed in Fig. 1B, only low concentrations of IL-37 were detected in normal arteries, where it was mainly expressed in VSMCs. By contrast, abundant IL-37 concentrations were detected in calcified arteries (Fig. 1D). In addition, the IL-37-positive area was significantly greater in calcified arteries than in normal arteries (39.2 ± 14.9% vs. 3.7 ± 1.4%, $P < 0.01$) (Fig. 1E). We used anti-CD3, anti-CD68, and anti-α-SMA antibodies to identify T lymphocytes, macrophages and VSMCs, respectively, in the calcified arteries. The results showed that IL-37 (Fig. 1F) was mainly expressed by macrophages and VSMCs (Fig. 1G and H) but was also expressed by T lymphocytes (Fig. 1I).