Clinical Research

Relationship between interleukin-18 levels and characterization of atherosclerotic plaque and percutaneous coronary intervention

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Background Interleukin-18(IL-18) plays a key role in the development, progression and outcome of coronary artery disease and its complications. However, its variability relation to the characterization of atherosclerotic plaque and percutaneous coronary intervention are still unknown. **Methods** Fifty four patients with coronary artery disease [22 patients with stable angina (SA) and 32 patients with acute coronary syndrome (ACS)] were enrolled in this study. All patients underwent percutaneous coronary intervention (PCI). The stability of the plaques at the criminal vessels was assessed with analogical IVUS. Serum IL-18 levels were measured at the time points of 5 min before PCI, and 0h, 6h, 24h and 1month after PCI in all patients. **Results** ACS group consisted mainly of lipidic unstable plaques while SA group of fibrous stable plaques. Moreover, compared with those in SA group, eccentricity index (EI) and remodeling index (RI) were significantly higher in ACS group. Positive remodeling was seen in ACS group while negative or no remodeling in SA group. Further, serum IL-18 levels were significantly elevated in patients with ACS than those in SA group before PCI, increased at 0h, 6h, 24h after PCI (P<0.05)and were not significant different at 1 month after PCI from those before PCI. **Conclusions** There is significant difference in the composition and structural characteristics of atherosclerotic plaques between ACS and UA groups. PCI triggersd and enhances the inflammatory response in a short time. Serum levels of IL-18 are the predictors of progression of unstable plaque in atherosclerosis. Post-operative complications of PCI might be reduced by inhibiting IL-18. (***J Geriatr Cardiol* 2008;5:21-24**)

Key Words IL-18; coronary artery disease; acute coronary syndromes; IVUS; percutaneous coronary intervention

In recent years, more and more evidence has suggested that atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by increasing accumulation of lipids, cells (macrophages, T lymphocytes and smooth muscle cells[SMCs]), and extracelluar matrix.[1] Many serum biomarkers have been investigated to determine their utility as tools for predicting risk of cardiovascular disease. Interleukin-18 (IL-18), a proinflammatory cytokine correlated with the innate and adaptive immune mechanisms, plays an important role in the inflammatory response that contributes to atherosclerosis.[2] Increased IL-18 expression has been demonstrated to accumulate in human atherosclerotic plaque and results in its corruption.[3] Furthemore, baseline circulating IL-18 level was reported to be a strong indicator to predict future cardiovascular mortality.[4] However, it remains unclear the relationship between IL-18 levels and plaque characterization. Furthermore, IL-18 levels in relation to PCI have not previously been studied. Therefore, this study aimed to investigate whether elevated levels of IL-18 are associated with the characteristics of atherosclerotic plaques and measure pre-and post-operative serum levels of IL-18 to estimate their clinical value in patients with CAD.

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Patients and methods

Patients

Twenty-two SA and 32 ACS patients, including 19 UA, 2 NSTEMI and 11 STEMI, were randomly collected from a population of consecutive subjects referred for selective coronary angiography from the First Hospital of Xiamen between September 2006 and May 2007.There were 28 males and 26 females ,aged 61.0±7.5 years (mean±SD).Those who had significant heart failure, tuberculosis, malignant diseases, acute or chronic infection, autoimmunity diseases, and liver or kidney diseases were excluded.

All patients received basic treatment with aspirin, |Â-blocker, ACE inhibitor, statin and clopidogrel. The local research's ethical committee approved the study protocol and written informed consent was obtained from all patients.

Specimens

A history of diabetes, hypertension and smoking was obtained by self-report. Before the angiography procedure, fasting blood samples were collected in ethylenediamine tetra acetates (EDTA) and centrifuged. Blood samples were also collected at the time points of 0 hour, 6 and centrifuged.

The plasma was stored at -70℃.Lipid and lipoprotein levels were measured soon after the subjects were recruited. Total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol levels were measured using the Friedewald formula. Fasting blood-glucose was measured using the Friedewald formula.

From May 2007 the frozen plasma samples were thawed and used to quantify the levels of IL-18 using ELISA method (Gene Technology Valley Co, Ltd, Shenzhen, China).The within-run coefficients of variation were determined to be 3.1% at a mean of 42.7 pg/ml and 8.4% at a mean of 102.8 pg/ml; between-run coefficients of variation were 3.1% at 42.7 pg/mL and 7.9% at 56 pg/ml (*n*=7 each).

Intravascular ultrasound

IVUS acquisition All subjects underwent CAG. IVUS (V5.2-VH, 3.5F, 20HZ, Volcano Co, USA) was used to assess the atherosclerotic plaques in criminal vessels. After administration of intra-coronary nitrates, the IVUS catheter was introduced up to the distal coronary bed of the criminal vessels. Using an automated pullback device, the transducer was withdrawn at a continuous speed of 0.5mm/s until the ostium was seen.

IVUS-VH analysis With the help of analogical histotechnique the composition and structural characteristics were quantitatively analyzed automatically (calcified tissue is labeled as white, fibrous as green, fibrolipidic tissue as greenish-yellow, and necrotic core as red). Unstable plaque was defined as: ¢Ùthe dark area of hypoechoic (lipidic core) >1mm^2.¢Úthe ratio of lipidic core to plaque area >20%.¢Û the thickness of fibrotic cap <0.7mm and ¢Üplaque rupture or thrombosis. Those did not fall in the standards above were stable plaques.[5]

The most obvious stenosis areas were examined by IVUS and the reference areas were measured at the nearest proximal and distal segments judged to be free of plaque, and mean value was calculated. External elastic membrane area (EEMA), lumen cross-sectional area (LA), diameter of the thickest plaque (D)max, diameter of the thinnest contralateral plaque (D)min were also measured. Plaque area (PA), ratio of area stenosis (LAS), eccentric plaque index (EI) and remodeling index (RI) were calculated. PA = EEMA-LA, LAS = PA / EEMA × 100%, EI = (D-max D/min) Dmax. EI=0.5 is considered to be concentric plaque, and EI>0.5 is eccentric plaque. RI = EEMA of lesions area/the mean EEMA of proximal and distal vascular reference. RI>1.05 is considered to be positive remodeling, and RI <0.95 is negative remodeling and RI between 0.95 and 1.05 is no remodeling.[6]

Data analyses

Discrete variables are presented as counts and percentages. Continuous variables are presented as mean±SD values. Between two groups, data were analyzed by an independent t-test with the SPSS statistical program, version12.0. Between multiple groups, one-way ANOVA was adopted. A value of P <0.05 was considered significant.

Results

Characteristics of patients

The clinical and demographical characteristics of patients were shown in Table 1. There were no significant differences between the 2 groups regarding to age, sex, history of diabetes and serum lipids levels.

Table 1 Clinical and demographical features of patients in 2 groups

	SA Group(*n*=22)	ACS Group(*n*=32)	*P*
Age	51.6±9.1	48.2±10.4	0.396
Male/female	10/12	18/14	0.477
Diabetes mellitus£Ûn(%)£Ý	7(22.7%)	8(25.0%)	0.398
Hypertension£Ûn(%)£Ý	10(40.9%)	2(37.5%)	0.321
Smoking£Ûn(%)£Ý	7(18.2%)	10(21.9%)	0.442
Family history£Ûn(%)£Ý	3(22.7%)	6(28.1%)	0.327
TC (mmol/L)	4.33±1.93	4.66±1.68	0.790
TG (mmol/L)	1.62±0.34	1.73±0.38	0.265
HDL-C (mmol/L)	0.71±0.24	0.66±0.22	0.530
LDL-C (mmol/L)	3.91±0.29	3.87±0.31	0.850
Criminal Vascular			0.495
LAD (case)	10	12	
LCX (case)	8	9	
RCA (case)	4	11	

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LAD: left anterior descending branch; LCX: left circumflex branch; RCA: right coronary artery.

Plaque characteristics

The IVUS- VH characteristics of coronary plaque in SA group and ACS Group: patients in SA group were mainly having stable and fibrous plaques while patients in ACS group having unstable and lipidic plaques. EI, RI and the numbers of lipidic plaques and unstable plaques in ACS group were significantly higher than those in SA group (Table 2).

SA, UA, IL-18 levels and plaque characteristics

The comparison of IL-18 serum levels between stable plaques and unstable plaques: the serum level of IL-18 in unstable plaques were significantly higher than that in stable plaques (P<0.01, Table 3).

Table 3 Comparison of IL-18 level between stable and unstable plaque

Item	Patient with stable plaque(n=28)	Patient with unstable plaque(n=26)
IL-18 (pg/L)	60.9±10.5	96.8±11.2*
SA (case)	22	0*
ACS (case)	6	26*

*P £¼0.01,compared with stable plaque group

SA, UA and IL-18 levels

The serum levels comparison of IL-18 between SA group and ACS group before PCI: the serum levels of IL-18 were significantly higher in ACS group than those in SA group before PCI (P <0.05) (Table 4).

PCI and IL-18 levels

The peak levels of serum IL-18 at the points of 0h, 6h and 24h after PCI were significant higher than those at 5 min before PCI£¨P<0.05£©.There were no significant differences between the levels of serum IL-18 on the point of 1 month after PCI and those on the preoperative point (Table 5).

Table 4 Comparison of serum IL-18 level between patients with SA and ACS

Patient	n	IL-18(pg/ml)
SA	22	61.8±10.2b
ACS	32	102.9±11.6b

Values are expressed as means±SD.
Compared with SA group, b:*P* £¼0.05

Discussion

This study presents a comprehensive analysis of the variability of IL-18 levels in relation to plaque characteristics in patients with SA and ACS. Our investigation also established the significant changes in IL-18 levels in patients undergoing PCI. IL-18, mainly produced by monocytes/macrophages, is involved in the development and complications of human atherosclerotic plaques.[7] It can induce the creation of IFN-¦Ãwith the synergism of IL-12. These cytokines accumulate in atherosclerotic plaques when expressed and have been involved in the immunoinflammatory response that participate in both the size and the composition of the atherosclerotic lesion.[8] IFN-¦Ácould influence plaque vulnerability by reducing the thickness of the fibrous cap through inhibition of collagen synthesis.[9]

Therefore, inflammation may play a key role in the development and progression of atherosclerosis. Inflammatory response can promote the formation, rupture of unstable plaque and thrombosis which causes acute coronary syndrome.[10] The composition and structural characteristics of plaque may impact the stability of plaque. Rodrighez. Granillo et al[11] confirmed that unstable plaque mainly appears as follows: thin fibrous cap, obviously eccentricity, and larger lipid core. With the help of virtual histological IVUS (IVUS-VH) we found that patients in ACS group were mainly having unstable, lipid and eccentric plaques, while those in SA

Table 2 the IVUS features of coronary plaque in SA and ACS

Item	SA(n=22)	ACS(n=32)	P
Plaque eccentric index	0.42±0.16	0.88±0.15	0.044
Vascular remodeling index	0.75±0.12	1.12±0.11	0.038
Stable plaques£Ûn(%)£Ý	22(100.0%)	6(18.7%)	0.012
Unstable plaque£Ûn(%)£Ý	0	26(81.3%)	0.000

Table 5 Preoperative and postoperative serum levels of IL-18 in patients with SA and ACS

Time	SA group (n=22)	ACS group (n=32)
5min before PCI (pg/ml)	52.87±10.27	78.25±12.10
0h after PCI (pg/ml)	88.63±12.75*	120.34±11.87*
6h after PCI (pg/ml)	135.42±11.82**	197.23±12.59**
24h after PCI (pg/ml)	138.68±13.27**	210.87±12.38**
1m after PCI (pg/ml)	48.27±10.31	53.36±11.21

Compared with the preoperative *P<0.05; **P<0.01

group were mainly having stable, fibrous and concentric plaques. ACS mainly presents positive remodeling while SA mainly presents negative or no remodeling. Eccentric index (EI), remodeling index (RI) and the numbers of lipid and unstable plaques in ACS group were significantly higher than those in SA group. Therefore, the stability of plaque plays an important role in the development and progression in the ACS. In addition, our study found that the serum levels of IL-18 in ACS group were significantly higher than that in SA group and by IVUS-VH ,we also found that the serum levels of IL-18 in unstable plaques were significantly higher than that in unstable plaques. Thus it suggested that the serum levels of IL-18 may become a reference indicator to predict the unstable plaque of coronary heart disease.

In this study, the serum levels of IL-18 were significantly increased within 24 hours after PCI, but the serum levels of IL-18 after 1 month of PCI there was no significantly change compared with the preoperative value, indicating that the earlier inflammation reaction in coronary artery may be triggered and aggravated by PCI. The reason may be:¢Ùthe lesion of the vascular endothelial cells by PCI may attract and activate the inflammatory cells such as neutrophil and monocyte-macrophage. The inflammatory cells may secrete many kinds of inflammatory cytokines such as IL-18; ¢Ûembolizating substances in the blood, especially the plaque and necrosis of the lipid of atherosclerosis which were flown into the microcirculation may activate and aggregate platelets. It can start a series of inflammatory reactions, and then inflammatory cytokines would be secreted;¢Ûthe coronary blood flow temporarily blocked by the filling balloon may cause short myocardial ischemia, the myocardium could be reperfused when balloon pumped into deflation. So balloon angioplasty can be considered as a clinical model of ischemia/ reperfusion injury. Cytokines play an important role in the inflammation mediation in the ischemia/reperfusion injury.[12]

Animal experiments have shown that the inhibition of IL-18 activity can delay the development of atherosclerosis, reduce the content of inflammatory cells and lipids of plaque, increase the content of smooth muscle cells and collagen, and stabilize the plaque. [13] Some small-scale clinical trials have confirmed that the postoperative complications can be reduced by inhibiting the inflammatory response.[14] However, in clinical practice, whether inhibiting IL-18 is useful for ACS remains to be confirmed by evidence-based medical research.

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