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The −251A>T polymorphism of interleukin-8 is associated with longer mechanical ventilation and hospital staying after coronary surgery

Zanxin Wang [a], Jie Shao [b], Qinghua Zhou [c], Jianshi Liu [d], Yu Zhu [c], Jie Yang [b,*], Minxin Wei [a,*]

[a] Department of Cardiovascular Surgery, Tianjin Medical University General Hospital, Tianjin, PR China
[b] Department of Immunology, Tianjin Medical University, Tianjin, PR China
[c] Tianjin Lung Cancer Institute, Tianjin, PR China
[d] Department of Cardiac Surgery, Tianjin Chest Hospital, Tianjin, PR China

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ABSTRACT

Background: Cardiac surgery is associated with inflammatory responses that are known to affect its outcome. The present study was designed to define whether post-operative release of interleukin (IL)-6, 8 and tumor necrosis factor-alpha (TNF-α) is related to the presence of a certain allele in functional polymorphism and its relationship to clinical outcome after off-pump coronary artery bypass (OPCAB). *Methods:* One hundred and forty-five patients undergoing first time elective OPCAB were genotyped for IL-6(−174G>C), IL-8(−251A>T) and TNF-α(−308G>A) polymorphisms using polymerase chain reaction (PCR) and gene sequencing. Cytokine levels were measured in plasma samples taken before the operation and 4, 24 and 72 h postoperatively by suspension array system. *Results:* Levels of IL-6 and IL-8 increased significantly after OPCAB. Patients with IL-6−174GG and IL-8−251AA genotypes had higher post-operative circulating levels of IL-6 and IL-8, respectively. Logistic regression showed that IL-8−251AA genotype was an independent risk factor of ventilation time more than 1 day (OR = 11.80, 95% CI: 1.87–74.48) and hospital staying more than 14 days (OR = 38.00, 95% CI: 4.15–347.87) after surgery. *Conclusions:* OPCAB results in post-operative inflammatory responses. Genetic backgrounds alter the extent of inflammatory response and might relate to clinical outcome of OPCAB.

1. Introduction

CABG is associated with a systemic inflammatory response syndrome that are known to affect the outcome after cardiac surgery [1–4]. Inflammatory cells produce cytokines that mediate various stages of inflammation and are capable of stimulating cells such as smooth muscle cells, fibroblasts and endothelial cells. Many researchers focus on the role of cytokines, particularly IL-6, IL-8 and TNF-α, in the inflammatory response caused by CABG [5,6].

OPCAB via median sternotomy may result in fewer episodes of post-operative complications [7] and less myocardial injury [8]. Prospective randomized trials have shown that the relatively new OPCAB technique provides a comparable clinical outcome to the established cardiopulmonary bypass (CPB) technique that has been continuously refined over more than three decades [9,10]. It can reduce morbidity and mortality associated with CPB, and has resulted in a greater acceptance of OPCAB procedures among the cardiovascular community [11]. However, it is also accompanied with some inflammatory responses caused by surgical trauma, contact of blood with foreign surfaces and reperfusion injury.

The network of inflammatory mediators is a very complex one, involving various kinds of pro-inflammatory and anti-inflammatory factors; TNF-α, IL-1, IL-6, IL-8, etc. being the most influent pro-inflammatory mediators and IL-4, IL-10, IL-13 being important anti-inflammatory mediators. Most previous studies on post-operative inflammatory reaction in open heart surgeries were directed towards IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, etc. [12,13]. A multifactorial pilot study with a small sample was first carried out. After gathering many related article reports, it was noted that the values of serum IL-6 and IL-8 at different time points postoperatively was far greater than those preoperatively. TNF-α is widely known to be produced in the initiating stages of inflammatory reactions, and has significantly affected the development of the inflammation after cardiac surgeries [14]. As a result, IL-6, IL-8 and TNF-α were chosen as target cytokines in this present study.

Individual genetic background could theoretically modulate the magnitude of post-operative systemic inflammatory reaction and, thus, contribute to a greater or lesser propensity for complications. Cytokine polymorphisms have been related with development of this process [15–17]. The present study was designed to define whether IL-6, IL-8 and TNF-α release in response to OPCAB is related to the presence of a certain allele in functional polymorphism and its relationship to clinical outcome.

* Corresponding authors. Fax: +86 22 60363501.
E-mail address: minxinw@yahoo.com (M. Wei).

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2. Patients and methods

2.1. Study patients

One hundred and forty-five patients undergoing first-time OPCAB were invited to participate in the study. The investigation was approved by the local ethics committee and informed written consent was obtained from all patients who entered the study. Patients with liver or renal failure, preexisting autoimmune disease, or severe infection were not eligible. Patients on immuno-suppressive therapy or anti-inflammatory agents were also excluded. Antiplatelet therapy was routinely stopped 5–7 days before surgery.

2.2. Anesthesia and surgical protocol

All patients had premedication with morphine. Standardized OPCAB operation was undertaken via median sternotomy, anesthetic techniques were used with sufentanyl, midazolam and vecuronium. Esmolol was given where necessary to reduce the heart rate. Coronary grafting was performed on a beating, normothermic heart with internal thoracic artery (ITA), and/or radial artery, and/or peripheral vein grafts from the lower extremities. A U-shaped stabilizer (Octopus®, Medtronic Inc., Minneapolis, MN, USA) was used to dampen the movement of the beating heart and consequently to isolate the region for anastomosis. Proximal anastomosis of the aorta was performed using tangential clamping. Heparin was administered after harvesting the internal mammary artery to achieve systemic anticoagulation during surgery by an initial dose of 1.5 mg/kg body weight. Activated clotting time (ACT) was adjusted to a target of over 300 s. ACT was measured using a kaolin-activated system (Medtronic Inc., Minneapolis, MN, USA). After the end of grafting procedure, the effect of heparin was reversed by protamine administration (1:1 ratio) to achieve an ACT to around 150 s. Post-operative treatments in ICU were standardized.

2.3. Cytokines assay

Citrated 2 ml blood samples were initially drawn before surgery and then again at 4, 24 and 72 h after OPCAB. These were immediately centrifuged (3000 rpm, 10 min), and plasma was separated and frozen at −80 °C until analysis. IL-6, IL-8 and TNF-α concentrations were measured by using a suspension array system (Liquichip, Qiagen, USA and Beadlyte® Human Multi-cytokine Detection System2; Beadlyte® Human Serum Diluent Pack, Millipore, USA) by a staff blinded to all subject data. Assay sensitivity was 0.2–3200 pg/ml.

2.4. Genotyping protocol

At recruitment, a 2 ml EDTA sample of peripheral blood was drawn, from which DNA was extracted by using Gentra DNA Isolation Kit (USA). Sequence amplification was performed by using PCR. IL-6(−174G>C), IL-8(−251A>T) and TNF-α(−308G>A) genotyping were performed following a methodology previously described [18,19].

2.5. Statistical analysis

Statistical analysis was performed using SPSS/Win (Version 13.0). Data are presented as median (quartiles) or mean ± standard deviation if appropriate. The χ^2 test was used to analyze relationship between categorical data. Nonparametric Mann–Whitney *U* test and Kruskal–Wallis test, as appropriate, were used to compare different outcomes between subgroups. The variation over time of cytokines was analyzed using analysis of variance for repeated measurements. Logistic regression was used to analyze the risk factors of outcome. Significance was established at a *P* value less than 0.05.

3. Results

One hundred and forty-five patients undergoing elective OPCAB were recruited. Average age was 63.0 ± 8.5 years old. All planned procedures were completed, and the operative time was 222.6 ± 47.8 min. The distal anastomoses were 2.7 ± 0.8. There were no deaths or major complications (cerebral complications, renal or hepatic dysfunction). Some patients whose genotype samples were lost had to be excluded. Genotype distributions were in Hardy–Weinberg equilibrium for all three polymorphisms. Allele frequencies are shown in Table 1.

Levels of IL-6 and IL-8 both increased after OPCAB, peaking at 4 h (IL-6: 118.5[53.9, 189.7] pg/ml; IL-8: 18.0[8.4, 37.1] pg/ml) and remaining above preoperative levels until 72 h after surgery. Trace perioperative levels of TNF-α were detected, and there was no difference in different time points (data no shown).

Adjusted for gender, age, smoking, drinking, hypertension, Type II diabetes mellitus, previous cerebrovascular disease, previous chronic lung disease, previous myocardial infarction, LVEF, diseased vessels, left main narrowed, operation time, distal anastomoses and blood loss, none of the variables of traditional risk factors differed significantly in IL-6−174GG/GC, IL-8−251AA/AT/TT, or TNF-α−308AG/GG genotypic groups ($P > 0.05$). Therefore, the clinical outcome can be more specifically associated with genetic background. Genetic analysis of IL-6(−174G>C) and IL-8(−251A>T) revealed that there was a significant effect of genotypes on cytokine levels over time. Patients with IL-6−174GG genotypes had higher post-operative circulating levels of IL-6 as compared to those with GC genotype. Patients with IL-8−251AA had higher IL-8 levels than those with T allele. The largest difference between groups was observed 4 h after OPCAB (IL-6: GG, 119.2[61.1 190.9] pg/ml, GC, 38.9[24.7, 50.8] pg/ml; IL-8: AA, 33.1[16.6, 49.5] pg/ml, AT, 18.3[8.8, 43.1] pg/ml, TT, 16.9[7.0, 27.8] pg/ml) (Fig. 1). As only trace TNF-α was detected in peripheral blood, its relationship with TNF-α genotype could not be analyzed.

Statistical comparison between genotypes and post-operative outcome illustrated that only IL-8−251AA had significantly higher rate of post-operative ventilation >1 day (50%) and post-operative stay >14 days (89%) (Table 2). Logistic regression identified IL-8−251AA genotype as an independent risk factor of ventilation time more than 1 day (OR: 11.80, 95% CI: 1.87–74.48) and post-operative hospital staying more than 14 days (OR: 38.00, 95% CI: 4.15–347.87). The result remained statistically significant after risk adjustment to traditional risk factors: gender, age, smoking, drinking, hypertension, diabetes mellitus, previous cerebrovascular disease, previous chronic lung disease, recent myocardial infarction, left ventricular ejection fraction (LVEF), diseased vessels, left main disease, operation time, distal anastomoses and blood loss (Table 3).

4. Discussion

Inflammation is involved in all stages of atherosclerotic development. It is a cause and consequence of ischemic heart disease

Table 1
Genotype distributions.

	n	Genotype		
IL-6(−174G>C)	144	GG(140)	GC(4)	CC(0)
IL-8(−251A>T)	145	AA(15)	AT(81)	TT(49)
TNF-α(−308A>G)	111	AA(0)	AG(18)	GG(93)