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Tobacco smoking and cytokine levels in human epicardial adipose tissue: Impact of smoking cessation

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ABSTRACT

Background & aims: Epicardial adipose tissue (EAT) is a source of a number of cytokines which could act in the pathogenesis of coronary artery disease (CAD). The potential relationship between known cardiovascular risk factors, such as smoking, dyslipidaemia or diabetes mellitus and EAT humoral signalling, has not been fully elucidated. Therefore, we designed and conducted a cross-sectional study to determine whether selected cardiovascular risk factors are linked to levels of cytokines in epicardial and subcutaneous adipose tissue (SAT).

Methods: Samples of SAT and EAT were collected from consecutive patients undergoing scheduled cardiac surgery. Tissue concentrations of tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), adipocyte fatty acid-binding protein, leptin, and adiponectin were determined by ELISA.

Results: We enrolled 140 patients. TNF-α and IL-6 concentrations in EAT and SAT were significantly higher in current smokers (CS) than in never smokers (NS) and former smokers (FS). There were no differences between FS and NS. No other clinical variables were associated with cytokine concentrations in a regression analysis.

Conclusions: Smoking was independently associated with higher TNF-α and IL-6 concentrations in EAT and SAT. A novel observation that pro-inflammatory cytokines are elevated in EAT in smokers could contribute to identify potential mechanisms involved in the pathogenesis of adverse effects of tobacco smoking. There were no differences between EAT cytokine production in NS and FS, which support the importance of smoking cessation for cardiovascular risk reduction.

1. Introduction

Epicardial adipose tissue (EAT) is a source of various pro- and anti-inflammatory cytokines and their levels may be markedly different from those observed in subcutaneous adipose tissue (SAT) [1]. Studies to date suggest that EAT pro-inflammatory profile, measured by concentrations of various cytokines, their mRNA, or pro-inflammatory cells could act in the pathogenesis of coronary artery disease (CAD) [2–4], or degenerative heart valve diseases [5]. Similarly, EAT inflammation studied by fluorodeoxyglucose positron emission tomography was linked to the occurrence of atrial fibrillation (AF) [6], and protective effect of EAT adiponectin on the development of postoperative AF has been shown [7]. A potential relationship between known cardiovascular risk factors and EAT humoral profile (measured by tissue levels of cytokines) has not been fully elucidated. In addition to these findings, the importance of smoking cessation has been extensively studied in recent years with convincing clinical results, however, the underlying mechanisms still remain unclear [8–10]. We conducted a cross-sectional study to determine the association between cardiovascular risk factors (in particular tobacco smoking status) and levels of selected cytokines in subcutaneous and epicardial adipose tissue.

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2. Materials and methods

Consecutive patients undergoing scheduled cardiac surgery in our Centre, who provided written informed consent, were enrolled in the study. All the patients underwent a comprehensive, routine, preoperative evaluation, which ruled out possible infectious foci or presence of acute infection. This assessment included laboratory evaluation of blood ions, urea, creatinine, glucose, C-reactive protein (CRP) and full blood count; dental and urological or gynaecological examination and pulmonary function testing. Patients with a documented history of autoimmune systemic inflammatory disorders were excluded. The smoking status of patients was assessed on admission by the admitting physician. Never smokers (NS) were defined as individuals who had not smoked more than 100 cigarettes in their lives; former smokers (FS) were those who had smoked more than 100 cigarettes but were not currently smoking, and current smokers (CS) were defined as patients who had smoked more than 100 cigarettes in their lives and were still smoking [11]. Dyslipidaemia and diabetes mellitus were diagnosed in accordance with current guidelines [12,13]. Relevant clinical and demographic characteristics were extracted from patients' medical records kept at our institution. The study was approved by the Institutional Review Board.

3. Adipose tissue collection and treatment

Adipose tissue biopsy samples were obtained immediately after mid-sternotomy before the initiation of cardiopulmonary bypass. EAT samples (1.0–2.0 g) were harvested near the proximal left and right coronary arteries and SAT samples were obtained from the sternotomy site from the chest wall. The specimens were rinsed with 0.1 M Tris (pH = 7.4) to remove blood. Frozen tissue samples were powdered in mortar chilled with liquid nitrogen and proteins were extracted for 20 min at room temperature, according to a standard protocol, with buffer containing detergent (3% TRITON X-100, 0.1 M Tris, pH 7.4) and protease inhibitor cocktail. After centrifugation (15 min, 4 °C, 10,000g), tissue extracts were aliquoted and stored at −80 °C until analysis.

4. Biochemical analysis

The selected markers included tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), adipocyte fatty acid-binding protein (AFABP), leptin, and adiponectin. Their concentrations in EAT and SAT sample extracts were determined by ELISA (BioVendor - Laboratorni medicina). Total protein concentration was determined by bicinchoninic acid assay (Sigma-Aldrich). Concentrations of the markers were adjusted for total protein concentration in the individual tissue samples.

5. Statistical analysis

Categorical variables are presented as absolute and relative frequencies. Continuous variables are displayed as mean ± standard error of the mean (SE). $p < 0.05$ was considered significant. One-way ANOVA was used to compare across 3 groups defined by patients' smoking status. If significant p value was observed, ANOVA was followed by the Tukey *post hoc* test, which protected the overall error rate. Categorical variables were compared across the three groups using the Chi Square test followed by the Marascuilo *post hoc* test. Stepwise linear regression analysis was used to adjust for effects of other clinical variables on SAT and EAT levels of individual markers. Previously described or *a priori* selected factors were included in the analysis: age, gender, body mass index (BMI), diabetes mellitus (DM), smoking, dyslipidaemia, hypertension and chronic use of statins, aspirin, and non-steroidal anti-inflammatory drugs [14]. Smoking status was arbitrarily included in the model, while the other confounders were selected using a stepwise method. Smoking entered the model as a dummy variable. Correlations were assessed by Pearson's r.

6. Results

Samples from 140 patients were obtained. Table 1 details the clinical and demographic characteristics of the patient groups. Information on smoking status was available in 132 patients of whom 76 (57.6%) were never smokers, 34 (25.8%) former smokers and 22 (16.7%) current smokers. Current smokers were significantly younger than never smokers ($p < 0.0001$) and former smokers ($p = 0.0222$). No significant difference was observed between never and former smokers. There were significantly more female patients among never smokers as compared to former smokers ($p < 0.0001$), but no difference in gender was seen between never and current smokers ($p = 0.1482$) and between former and current smokers

Table 1
Characteristics of the enrolled patients.

	All patients (n = 140)	Never smokers (n = 76)	Former smokers (n = 34)	Current smokers (n = 22)	p value (ANOVA or Chi square)
Gender (female)	44 (31.4%)	33 (43.4%)	1 (2.9%)	5 (22.7%)	<0.0001
BMI	29.2 ± 0.4	29.6 ± 0.5	29.0 ± 0.7	27.4 ± 0.9	0.1337
Age	68.0 ± 0.9	70.4 ± 1.1	66.5 ± 1.6	59.5 ± 2.0	<0.0001
Diabetes mellitus	53 (37.9%)	27 (35.5%)	14 (41.2%)	8 (36.5%)	0.85
COPD	27 (21.8%)	10 (15.2%)	8 (25.0%)	8 (38.1%)	0.0861
Dyslipidaemia	97 (69.3%)	49 (64.5%)	26 (76.5%)	18 (81.8%)	0.1957
CRP mg/L	6.9 ± 0.9	5.5 ± 1.2	8.1 ± 1.8	7.4 ± 2.4	0.4262
Coronary artery disease	90 (64.3%)	44 (57.9%)	27 (79.4%)	15 (68.2%)	0.0771
Type of surgery					
CABG	63 (45.0%)	28 (36.8%)	19 (55.9%)	13 (59.1%)	0.0667
AVR	43 (30.7%)	28 (36.8%)	7 (20.6%)	6 (27.3%)	0.2149
CABG + AVR	27 (19.3%)	16 (21.1%)	8 (23.5%)	2 (9.1%)	0.3737
Other surgery	7 (5.0%)	4 (5.3%)	1 (20.6%)	0	0.4011
LVEF (%)	54.7 ± 1.0	57.4 ± 1.3	50.2 ± 1.9	52.3 ± 2.4	0.0052
LA diameter (mm)	41.7 ± 0.5	41.4 ± 0.6	41.9 ± 0.9	40.8 ± 1.2	0.7279
Statin	94 (67.6%)	47 (61.8%)	25 (75.8%)	18 (81.8%)	0.1135
Aspirin	67 (48.5%)	37 (46.9%)	15 (46.9%)	13 (59.1%)	0.6361
NSAID	6 (4.3%)	4 (5.3%)	1 (3.0%)	0	0.3395

Categorical variables are shown as counts and percentages in the groups defined by smoking status.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; CRP, serum C reactive protein; CABG,coronary artery bypass graft; AVR, aortic valve replacement; LVEF, left ventricular ejection fraction; LA, left atrium; NSAID,non-steroidal anti-inflammatory drugs.