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C-reactive protein (+1444C > T) polymorphism influences CRP response following a moderate inflammatory stimulus

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Abstract

Elevations in C-reactive protein (CRP) concentration are associated with an increased risk of future coronary events in prospective studies and it has been suggested that CRP could be used to aid risk prediction. A +1444C > T polymorphism in the CRP gene has been associated with differences in CRP concentration. We investigated the effect of this polymorphism on the CRP response to periodontal therapy, an intermediate inflammatory stimulus. Clinical parameters, CRP, and interleukin-6 (IL-6) concentrations were evaluated in 55 consecutive patients suffering from periodontitis at baseline, 1, 7 and 30 days after an intensive course of periodontal treatment. In a multivariate analysis individuals homozygous for the +1444T allele showed higher CRP concentrations (day 1, 21.10 ± 4.81 mg/L and day 7, 4.89 ± 0.74 mg/L) compared with C-allele carriers (day 1, 12.37 ± 1.61 mg/L and day 7, 3.08 ± 2.00 mg/L). This effect was independent of conventional cardiovascular risk factors and inflammatory factors known to affect CRP concentrations. CRP genotype may need to be considered when CRP values are used in coronary risk prediction.

Keywords: Periodontitis; Inflammation; Polymorphism; Genetics; C-reactive protein

1. Introduction

Inflammation plays an important role in the pathogenesis of atherosclerosis [1]. Prospective studies indicate a robust and strong association, in healthy individuals and subjects with pre-existing atherosclerosis, between levels of C-reactive protein (CRP) and later cardiovascular events [2,3]. The CRP rise seen during acute coronary syndromes is also predictive of adverse outcome in the short term [4]. Therefore, it has been proposed that measurement of CRP may be a useful adjunct to standard coronary risk assessment [5]. The sensitivity of CRP to acute intercurrent inflammatory stimuli together with its wide dynamic range (0.1–1000 mg/L) render it an excellent clinical marker of infective or inflammatory episodes but represent a challenge to its clinical use in coronary risk prediction [6]. Understanding the factors that regulate CRP release at baseline and during infection or inflammation is therefore critically important in facilitating the correct interpretation of elevated CRP concentrations in the context of risk prediction. Although CRP might have an important role in the pathogenesis and prediction of coronary events, the factors, others than interleukin-6 (IL-6), influencing the basal and stimulated CRP concentrations achieved during acute inflammation in naturally occurring human models are incompletely understood. Twin and family studies indicate that CRP is a heritable trait [7,8]. We have recently identified a polymorphism (+1444C > T)

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in the 3′-untranslated region of the gene encoding CRP and demonstrated that in healthy subjects and coronary artery bypass graft (CABG) patients, homozygosity for the T allele was associated with higher basal and with stimulated CRP concentrations in male subjects [9]. However, it is likely that other important genetic influences such as, the −174G > C polymorphism of the IL-6 gene [10,11], as well as non-genetic influences may also determine the basal and stimulated concentrations of CRP during naturally occurring inflammation.

Intensive periodontal therapy causes a sharp rise in inflammatory markers (CRP and IL-6), peaking by 24 h after the intervention and sustained up to 1 week [12]. Periodontitis is a natural occurring prototype of chronic low-grade infection and inflammation [13], which has been also associated in prospective studies by an increased risk of cardiovascular events [14]. We therefore evaluated effect of the +1444C > T polymorphism on acute CRP release after intensive periodontal therapy and its dependence on the IL-6/−174G > C polymorphism and standard cardiovascular risk factors.

2. Methods

2.1. Study subjects

This study was conducted on 55 consecutive healthy individuals referred to the Department of Periodontology of the Eastman Dental Hospital, UCL, UK. Only subjects presenting with severe (probing pocket depths greater than 6 mm and marginal alveolar bone loss >30%, and generalized (at least 50% of teeth affected) periodontitis were invited to participate in the study. Exclusion criteria included known systemic diseases (hypertension, diabetes, dyslipidemia and history of myocardial infarction or stroke), history and/or presence of other acute or chronic infections, systemic antibiotic treatment in the preceding 3 months, treatment with any medication known to affect the serum level of inflammatory markers (e.g. statins, steroids, hormone replacement therapy) and/or pregnant or lactating females. All patients had given written informed consent; the study had been reviewed and approved by the Eastman/UCLH joint ethics committee.

2.2. Study protocol

A baseline visit was conducted to collect a complete medical history and standard clinical periodontal parameters (periodontal probing depths, recession of the gingival margin and clinical attachment levels). Subjects thereafter received an intensive session of subgingival mechanical instrumentation (including extraction of compromised teeth) under local anesthesia (within 6 h) as previously described [12]. During the study period (1 month follow-up), patients remained stable and there were no changes in lifestyle including exercise, diet, smoking or medications.

2.3. Inflammatory marker assays

Serial blood samples at baseline, 1, 7 and 30 days after periodontal therapy were obtained. Serum CRP concentrations were assessed by an automated immunoturbidimetric high-sensitivity assay (Cobas Integra, Roche AG Diagnostics, Mannheim, Germany) with a detection limit of 0.25 mg/L, and inter and intra-assay coefficient of variation of 4 and 5%, respectively. IL-6 was measured with high-sensitivity sandwich ELISA kits (Quantikine HS, R&D System, Minneapolis), detection limit 0.04 ng/L, and inter- and intra-assay coefficient of variation less than 5%. Laboratory measurements were carried out in a blind fashion and in single batches to limit inter-assay variability.

2.4. CRP and IL-6 genotyping

DNA was extracted by means of the QIAamp DNA blood minikit (QIAGEN©, Hilden, Germany) from dipotassium EDTA anticoagulated blood. The CRP/+1444C > T polymorphism was genotyped by PCR and RFLP analysis using primer pairs described previously [9] and the restriction enzyme *Sdu*I which cleaves the 181 bp PCR product into 23 and 158 bp fragments only in the presence of the common C allele. The IL-6/−174G > C polymorphism was genotyped by PCR and RFLP analysis using primer pairs described previously [15], and the restriction enzyme *Nla*III which cleaves the 190 bp PCR product into 143 and 47 bp in the presence of the rare C allele. All DNA analysis was performed by staff blinded to the clinical status of the patients.

2.5. Statistical methods

Preliminary analysis of normality was performed using the Shapiro–Wilk test. For CRP and IL-6, because of the skewed distribution, logarithmic transformations were used and data are reported as geometric mean ± standard deviation (S.D.). Changes in serum concentrations of CRP following periodontal therapy were used as the outcome variable in a one-way ANCOVA analysis adjusting for potential confounders: age, gender, ethnicity, body mass index (expressed in kg/m^2), smoking, blood pressure, periodontal diagnosis, number of teeth extracted and IL-6. Furthermore, a repeated measures ANOVA analysis was also performed to determine the effect of the (CRP/+1444C > T and IL-6/−174G > C) polymorphisms on the time course of inflammatory markers, after adjustment for potential confounders. For these analyses, recessive (CRP/+1444C > T) and dominant (IL-6/−174G > C) genetic models were used as a priori hypotheses [9]. Post hoc analyses were performed with Bonferroni corrections. A χ^2-test was used to compare genotype frequencies according to the Hardy–Weinberg equilibrium. Linkage disequilibrium between CRP/+1444C > T and IL-6/−174G > C polymorphisms was estimated using the method of Chakravarti et al. [16]. Data were analyzed with the statistical software package SPSS (SPSS Version 11, Chicago, IL).