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Original Contribution

Oxidative stress levels are raised in chronic fatigue syndrome and are associated with clinical symptoms

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Abstract

The aetiology of chronic fatigue syndrome (CFS) is unknown; however, recent evidence suggests excessive free radical (FR) generation may be involved. This study investigated for the first time levels of 8-iso-prostaglandin-$F_{2\alpha}$-isoprostanes alongside other plasma markers of oxidative stress in CFS patients and control subjects. Forty-seven patients (18 males, 29 females, mean age 48 [19–63] years) who fulfilled the Centres for Disease Control classification for CFS and 34 healthy volunteers (13 males, 21 females, 46 [19–63] years) were enrolled in the study. The CFS patients were divided into two groups; one group had previously defined cardiovascular (CV) risk factors of obesity and hypertension (group 1) and the second were normotensive and nonobese (group 2). Patients had significantly increased levels of isoprostanes (group 1, $P = 0.007$; group 2, $P = 0.03$, unpaired t test compared to controls) and oxidised low-density lipoproteins (group 2, $P = 0.02$) indicative of a FR attack on lipids. CFS patients also had significantly lower high-density lipoproteins (group 1, $P = 0.011$; group 2, $P = 0.005$). CFS symptoms correlated with isoprostane levels, but only in group 2 low CV risk CFS patients (isoprostanes correlated with; total symptom score $P = 0.005$; joint pain $P = 0.002$; postexertional malaise $P = 0.027$, Pearson). This is the first time that raised levels of the gold standard measure of in vivo oxidative stress (isoprostanes) and their association with CFS symptoms have been reported.

Keywords: Oxidative stress; Chronic fatigue syndrome; Free radicals; Cardiovascular risk factors

Introduction

Chronic fatigue syndrome (CFS) is a condition characterised by debilitating fatigue and other nonspecific symptoms resulting in significant disability, and its pathophysiology continues to remain elusive. A number of biological systems have been implicated and there is mounting evidence that oxidative stress [1–5] and, more specifically, lipid peroxidation contribute to the disease process [6] and to some of the symptoms in the illness [1]. Oxidative stress has been defined as a disturbance to the equilibrium status of prooxidant and antioxidant systems in favour of prooxidation. The term oxidative stress is used to describe a number of chemical reactions involved in the production of free radicals and other reactive molecules that are potentially able to induce cellular injury.

While free radicals may generate tissue oxidative injury it is also evident that other oxidative by-products, especially peroxidised lipids such 8-iso-prostaglandin $F_{2\alpha}$, may be even more pivotal in the pathological process. 8-Iso-prostaglandin $F_{2\alpha}$ is a member of the F_2-isoprostane family and can exert potent biological activity, such as platelet activation, and act as a powerful vasoconstrictor of the peripheral vasculature [7,8]. Such biological effects may be instrumental in the development of some of the vascular features that characterise patients with CFS [9,10].

Abbreviations: CFS, chronic fatigue syndrome; CV, cardiovascular; oxLDL, oxidised low-density lipoproteins; HDL, high-density lipoproteins; CDC, Centres for Disease Control; CVD, cardiovascular disease; BMI, body mass index; MAP, mean arterial pressure; GSH, glutathione; LDL, low-density lipoprotein; NF-κB, nuclear factor-kappa B; HSV, 1-herpes simplex virus type 1.

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A further indication of the in vivo consequences of increased lipid peroxidation would be higher levels of oxidised low-density lipoproteins (oxLDL) accompanied by low levels of high-density lipoproteins (HDL), which are associated with the development of atherosclerosis [11]. This study set out to investigate, for the first time, levels of 8-iso-prostaglandin $F_{2\alpha}$ alongside other markers of oxidative stress and antioxidant status in well-defined CFS patients and comparable control subjects, and to relate these levels to reported clinical symptoms of CFS.

Subjects and methods

Fifty-four patients were recruited from a register of several hundred local CFS patients. After a medical examination 47 patients (19 males and 28 females, mean age 48 years [19–63 years], 6 current, 3 ex-, and 38 nonsmokers) were found to fulfil the Centres for Disease Control (CDC) classification for CFS [12]. Seven of the patients were excluded: one had diabetes, one had a possible neurological condition underlying the fatigue, one had angina, two patients were unable to undertake the tests, and two did not fulfil the criteria for CFS. The mean length of illness was 9.2 (SD 5.7) years. All patients scored their CFS symptoms (the main 8 Fukuda et al. [12] CFS symptoms) [muscle pain, joint pain, headaches, postexertional malaise, sore throat, tender lymph nodes, headaches, unrefreshing sleep] as either absent (0), mild (1), moderate (2) or severe (3). Hypertension and obesity are both risk factors for cardiovascular disease (CVD), and therefore we divided the CFS patients into two groups for the analyses, low and high risk.

Thirty-four healthy volunteers who were matched (to the patients) for sex, age, and smoking status (13 males and 21 females, 46 [19–63] years, 5 current, 2 ex-, and 27 nonsmokers) were also recruited.

The local medical ethics committee approved the study which was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Each volunteer gave written informed consent to take part. One physician (CU) examined all patients. Height and weight measurements were recorded for all subjects and body mass index (BMI) was calculated. Blood pressure measurements were taken both supine and immediately upon standing and the mean arterial pressure (MAP) was calculated using the formula; MAP = [(2 × diastolic) + systolic] / 3.

A 15-ml blood sample was taken from the antecubital fossa and collected into tubes containing EDTA. All blood samples were taken at the same time of day. The blood was centrifuged for 15 min at 3500 rpm at 4°C, and plasma was then removed, aliquoted, and stored at −70°C until assayed for isoprostanes, oxLDL, cholesterol, and HDL. The buffy coat was removed and the remaining red pellet from the 10-ml EDTA blood tube was washed three times with normal saline (0.9% sodium chloride solution). This resulted in a pellet of packed red blood cells. Levels of the antioxidant glutathione (GSH) were measured on a spectrophotometer from the packed red blood cells by the method of Ellman [13]. Plasma isoprostanes were measured by gas chromatography-mass spectrometry following the method described by Roberts and Morrow [14]. Total cholesterol and HDL levels were measured on a Cobas Bio centrifugal analyser using products from Roche. Plasma oxLDL levels were measured by ELISA (Mercodia, Sweden).

Results

CFS patients in group 1 (CVD risk factor group) [n = 16; 5 males and 11 females, mean age 52.4 years (35–62 years)] were obese (BMI >30) and hypertensive, as defined by the European Society of Hypertension [systolic >140 mm Hg, or diastolic >90 mm Hg] [15]. CFS patients in group 2 [n = 31; 14 males and 17 females, mean age 46 years (19–64 years)] were normotensive and had a BMI of <30. Each patient group was compared with sex- and age-matched controls. Four of the controls had hypertension and they were placed in the control group which matched patient group 1 (CVD risk factor group). At the time of study none of the subjects with hypertension were on any medication to lower BP, although this was subsequently addressed.

The data were normally distributed and an unpaired *t* test was used to compare the mean levels of all the parameters between the groups. Pearson correlation coefficient was used as a measure of linear association.

CFS patient group 1 (CVD risk factor group) had statistically significantly increased BMI ($P < 0.001$), supine blood pressure (systolic $P = 0.049$, and diastolic $P = 0.023$), supine MAP ($P = 0.016$), and 8-iso-prostaglandin $F_{2\alpha}$-isoprostanes ($P = 0.007$), and significantly lower HDL ($P = 0.011$) and GSH ($P = 0.023$) when compared with their control group (Table 1).

CFS patient group 2 (CVD low-risk factor group) had statistically significantly higher levels of oxLDL ($P = 0.02$) and 8-iso-prostaglandin $F_{2\alpha}$-isoprostanes ($P = 0.03$) and significantly lower HDL levels ($P = 0.005$) than their matched control group (Table 2).

Cholesterol levels were not significantly different between either of the patient groups and their controls.

To assess symptom severity we used the CDC 1994 (Fukuda) severity score that we derived for each symptom [16]. We found that for the CFS patients who were normotensive and nonobese, (CFS patient group 2), 8-iso-prostaglandin $F_{2\alpha}$-isoprostane levels significantly positively correlated with joint pain (correlation Pearson coefficient $r = 0.546$, $P = 0.002$), postexertional malaise ($r = 0.411$, $P = 0.027$). Both muscle pain and unrefreshing sleep showed a pattern of association, but this did not reach statistical significance ($r = 0.337$, $P = 0.074$ and $r = 0.338$, $P = 0.073$, respectively).