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Short Communication

Inflammatory activation and cholinergic anti-inflammatory system in eating disorders

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ABSTRACT

Dysfunctional serotoninergic regulation and hypothalamic–pituitary–adrenal (HPA) axis overreactivity have been consistently reported in research studies with eating disorders (ED). In addition, the links between stress response, serotonin function, HPA axis and inflammatory mechanisms in ED have also been suggested in a number of studies. In our study, inflammatory parameters in white blood cells were investigated in 26 female patients with ED and 25 healthy control subjects matched for sex, age and ethnicity. Patients were free of medication for at least two weeks at the time of the study. Results showed a significant increase in plasma levels of the proinflammatory cytokine IL1β and the protein expression of cyclooxygenase 2 (COX2) in peripheral mononuclear blood cells (PMBCs) in ED patients compared with controls. As well as a significant increase of the oxidative-nitrosative marker TBARS (Thiobarbituric Acid Reactive Substances) in plasma. These findings were associated with increased expression of the alpha7 subunit of the nicotinic receptor (α7nAChR) in PMBC in ED patients independent of plasma cotinine levels.

These results suggest that a pro-inflammatory and oxidant phenotype might be present in ED patients. Further research on cellular inflammatory and anti-inflammatory pathways might be oriented to investigate differences between ED subtypes and to search for new potential targets for pharmacological treatment.

1. Introduction

DSM-IV criteria for Anorexia Nervosa (AN) include inadequate weight lost, an intense fear of gaining weight or becoming fat, a refusal to maintain normal weight for age and height, and amenorrhea. Binge-eating or purging behavior is present in the Binge Eating/Purging type but not in the restrictive type of AN. Bulimia Nervosa (BN) is characterized for recurrent episodes of binge eating and inappropriate compensatory behavior such as fasting, purging or exercise. Finally Eating disorder not otherwise specified (ED-NOS) might be described as an eating disorder that does not meet the criteria for AN or BN (American Psychiatric Association, 2000).

Etiopathogenesis of eating disorders is still not fully known, and data about possible biomarkers are scarce. Alterations in some neurotransmitters have been proposed (Crisp, 1978; Leibowitz, 1987) as well as genetic abnormalities (Scherag et al., 2010). Among neurochemical research studies on ED patients, dysfunctional serotoninergic regulation (Ribases et al., 2008) and hypothalamic–pituitary–adrenal (HPA) axis overreactivity (Basurte et al., 2004; Bruce et al., 2012) are the most consistently reported findings. Furthermore, the linkage between stress response, inflammation factors and anorexia nervosa has been proposed in several studies with eating disorders (Silva, 2011; Brambilla, 2001). Stress related sympathetic nervous system activation releases catecholamines, which in turn enhances production of proinflammatory mediators such as cytokines Tumor Necrosis Factor Alpha (TNFα) or Interleukin 1 beta (IL1β), nuclear transcription factors such as *kappa* B (NFκB) and Nitric Oxide (NO) (Lucas et al., 2006). These inflammatory and oxido-nitrosative mediators have been shown to be increased in brain and at peripheral level, in both animal (García-Bueno et al., 2008) and human models of stress (Bierhaus et al., 2003).

A number of studies have examined the inflammatory response in patients with eating disorders suggesting that significant

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changes at the cytokine levels are present in these patients. Elevated concentrations of TNF-α, IL-6 (Ahren-Moonga et al., 2011; Brambilla et al., 1998; Emeric Sauval et al., 1989; Kahl et al., 2004; Mitchel et al., 1994; Nakai et al., 1999; Pomeroy et al., 1994;) and IL-1β (Nova et al., 2002) have been reported. Some authors have proposed a "cytokine hypothesis" for ED based on the presentation of symptomatic anorexia following inflammatory conditions or fever syndromes (Corcos et al., 2003; Dantzer, 2004). However, other studies have reported negative findings in eating disorders (Brambilla, 2001; Monteleone et al., 1999; Polack et al., 1993; Schattner et al., 1990a,b).

Regulation of inflammation includes both inflammatory and anti-inflammatory processes. Two major anti-inflammatory mechanisms are: (1) cyclopentenone prostaglandins derived from the differential activation of cyclooxygenase (COX) isoforms by different physio-pathological stimuli, and (2) the activation of the alpha7 subunit of the nicotinic receptor (α7nAChR), which has been implicated in controlling the NFκB-mediated inflammatory mechanisms (Altavilla et al., 2006).

Based on data obtained in experimental stress models and in clinical studies in patients with eating disorders (Corcos et al., 2003; Nova et al., 2002; Raymond et al., 2000) we have tested the hypothesis of a disruption of the physiological equilibrium of proinflammatory/anti-inflammatory pathways in ED. Particullarly, we have explored a group of intracellular and soluble components of inflammatory and oxidative response in patients and healthy controls.

2. Methods

2.1. Sample

Twenty-five female patients with current diagnosis of eating disorder (AN, BN or ED-NOS) were included in the study. Subjects were selected at the Eating Disorders Unit of a general hospital and were evaluated with structured interviews for mental disorders (SCID-I) and for personality disorders (SCID-II) by a senior psychiatrist. To further characterize the psychopathology of the disorder we used different eating disorders assessment tools including the Eating Disorders Inventory (EDI) (Garner et al., 1983), the Bulimic Investigatory Test Edinburgh (BITE) (Henderson and Freeman, 1987), and the Body Shape Questionnaire (BSQ) (Cooper et al., 1987).

Mean age of patients was 25 years (±5.95). Nine patients were AN (five Restrictive type and four purging type), 14 patients were BN, one patient was ED-NOS and two were diagnosed as Binge eating disorder. Scores in the tests for eating disorders (BITE, EDI and BSQ) are shown in Table 1. Body Mass Index (BMI) of patients varied between 37.26 and 15.06 kg/m^2, and the mean value was 20.28 kg/m^2 (±SD 5.72). The BMI mean value of impulsive patients was 21.10 kg/m^2 (±SD 6.43) and of restrictive patients was 17.85 kg/m^2 (±SD 1.53). BMI for controls was 23.3 kg/m^2 (19.5–25 kg/m^2).

Patients were selected consecutively during a period of six months and subjects with current major depression or chronic dysthymia, current substance dependence disorders and life history of schizophrenia and bipolar disorder were excluded from the study. Patients had been free of medication for at least two weeks at the time of the study.

The control group was selected among female personal staff of the hospital facilities and included 25 female subjects, matched for age and educational level with the patient group, and free of any current psychiatric disorder or medical disorder that could potentially affect inflammatory parameters. Control subjects were all evaluated with SCID-I structured interview by a senior psychiatrist and, besides current axis I disorders such as major depression, dysthymia or substance dependence disorders, lifetime history of schizophreniform or bipolar disorder was also considered as exclusive for entering the study. None of the control subjects included had full or subthreshold criteria for eating disorders, either restrictive or bulimic. The study was approved by the institutional ethical committee of the Hospital Clinico San Carlos (Madrid–Spain) and all participants signed written informed consent after receiving complete description of the study.

2.2. Blood collection

Blood samples (10 mL) were taken at approximately 8:30 a.m. after fasting overnight by venipuncture into heparinized tubes. Plasma was obtained by centrifuging at 1800 rpm for 10 min at 4 °C immediately after sample collection. All plasma samples were stored at −80 °C until assay.

Peripheral mononuclear blood cells (PMBC) were prepared for some determinations: the rest of the sample discarded after plasma removal was 1:2 diluted in culture medium (RPMI 1640, Lonza, Verviers, Belgium) and a gradient with Ficoll–Paque® (GE Healthcare, Uppsala, Sweden) by centrifugation (800 *g* 40 min, RT). PBMC layer was carefully aspired and resuspended in RPMI and centrifuged (1116 *g* 10 min, RT). The supernatant was removed and the mononuclear cell enriched pellet was stored at −80 °C until processing.

Determinations of transcription factors, its inhibitory subunits or nuclear receptors were carried out in cytosolic and/or nuclear extracts from PMBC (see below). For preparation of cytosolic fraction and nuclear extracts, a modified procedure based on Schreiber et al. (1989) was used.

2.3. Biochemical determinations

Several biomarkers for inflammatory activity were measured including:

a. Inducible isoforms of two major inflammatory enzymes: nitric oxide synthase (iNOS) and cyclooxygenase (COX-2), as well as soluble markers of its activity: NO2 (nitrites, the stable metabolites of NO) and prostaglandin E2 (PGE_2) respectively.
b. Soluble markers of oxido–nitrosative status: TBARS (Thiobarbituric Acid Reactive Substances) as an index of lipid peroxidation (by-products after the attack of reactive oxygen species to lipidic components of the cellular membranes)
c. Two of the main proinflammatory cytokines, IL1β and TNFα
d. Intracellular mechanisms controlling iNOS and COX-2 expression: nuclear transcription factor κB (NFκB).

On the other hand, components of the antiinflammatory pathway were also investigated, including:

a. The main cyclooxygenase derived anti-inflammatory product, 15 deoxy prostaglandin J2 (15d-PGJ_2),
b. Its nuclear target, a transcription factor inhibiting gene expression and synthesis of inflammatory mediators: peroxisome proliferator-activated receptor gamma (PPARγ)
c. An alternative anti-inflammatory mechanism, the α7 subunit of the nicotinic receptor (α7nAChR), which has been implicated in controlling NFκB mediated inflammatory mechanisms in different experimental *in vivo* and *in vitro* settings (Altavilla et al., 2006).

2.4. Plasma measurements

Cytokine levels were measured by enzyme immunoassay using reagents in kit form TNFα (human) EIA kit, Interleukin-1β (human)