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Regulation of inflammatory pathways in schizophrenia: A comparative study with bipolar disorder and healthy controls

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ABSTRACT

Background: Immune-inflammatory processes have been implicated in schizophrenia (SCH), but their specificity is not clear.
Main aim: To identify potential differential intra-/intercellular biochemical pathways controlling immune-inflammatory response and their oxidative-nitrosative impact on SCH patients, compared with bipolar disorder (BD) patients and healthy controls (HC).
Methods: Cross-sectional, naturalistic study of a cohort of SCH patients ($n = 123$) and their controls [BD ($n = 102$) and HC ($n = 80$)].
Statistical analysis: ANCOVA (or Quade test) controlling for age and gender when comparing the three groups, and controlling for age, gender, length of illness, cigarettes per day, and body mass index (BMI) when comparing SCH and BD.
Results: Pro-inflammatory biomarkers: Expression of COX-1 was statistically higher in SCH and BD than HC ($P < 0.0001$; $P < 0.0001$); NFκB and PGE2 were statistically higher in SCH compared with BD ($P = 0.001$; $P < 0.0001$) and HC ($P = 0.003$; $P < 0.0001$); NLRP3 was higher in BD than HC ($P = 0.005$); and CPR showed a gradient among the three groups. Anti-inflammatory biomarkers: BD patients had lower PPARγ and higher 15d-PGJ2 levels than SCH ($P = 0.005$; $P = 0.008$) and HC ($P = 0.001$; $P = 0.001$). Differences between SCH and BD: previous markers of SCH (NFκB and PGE2) and BD (PPARγ and 15d-PGJ2) remained statistically significant and, interestingly, iNOS and COX-2 (pro-inflammatory biomarkers) levels were statistically higher in SCH than BD ($P = 0.019$; $P = 0.040$).
Conclusions: This study suggests a specific immune-inflammatory biomarker pattern for established SCH (NFκB, PGE2, iNOS, and COX-2) that differentiates it from BD and HC. In future, their pharmacological modulation may constitute a promising therapeutic target.

1. Introduction

Schizophrenia (SCH) is a severe, complex, multifactorial disorder that affects approximately 0.7% of the world population [1,2]. In recent years, there have been changes in the approach to SCH, with a focus on the search for biological markers [3–7]. In this sense, there is renewed interest in immune-inflammatory changes and their associated oxidative-nitrosative consequences as key pathophysiological mechanisms of the neuroprogressive pathways of this disorder [8].

Several hypotheses involving inflammatory processes caused both by external and endogenous factors have been implicated in SCH [8–11]. Inflammation is a complex biological protective mechanism, but when excessive in intensity or time, it becomes harmful. Intracellular events, such as cytoplasmic/-nuclear tran-

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scription factors, mainly kappaB (NFκB), control the expression of several oxidative and nitrosative mediators through activation of inducible enzymes, as key factors in this regulation. Furthermore, intercellular elements such as cytokines and chemokines are crucial elements of proper inflammatory response. Such a complex defense mechanism is finely regulated by compensatory anti-inflammatory pathways [12]. One of these mechanisms involves cyclopentenone prostaglandins (PGs) such as 15-deoxy-Δ12,14-PGJ2 (15d-PGJ_2) [13], one of the proposed endogenous ligands for the gamma isoform of peroxisome proliferator-activated nuclear receptors, PPARγ. The PPARγ is a transcription factor that mitigates inflammation by repressing the expression of pro-inflammatory cytokines and the inducible isoforms of COX and NOS: COX-2 and iNOS [14,15].

Early studies in SCH described elevations in plasma levels of pro-inflammatory cytokines [16,17] and decreases in anti-inflammatory cytokines [18]. However, recent studies focus on intra- and intercellular biochemical pathways controlling inflammatory response and found a systemic imbalance in some pro-/anti-inflammatory mediators in these patients [11]. Most of the imbalance studies have been carried out in early stages of the disease: subtle alterations in immune-inflammatory mediators and oxidative-nitrosative stress have already been found at disease onset [19,20]. In particular, there was an increase in levels of pro-inflammatory NFκB, iNOS and COX-2 in patients with a first episode of psychosis (FEP) compared with healthy controls (HC) [21] and of PGE2 in patients with established SCH [22]. Furthermore, the inhibitory subunit of NFκB, 15d-PGJ_2, and PPARγ expression and transcriptional activity were lower in FEP patients [21] along with anti-inflammatory PGs in peripheral monocytes in patients with established SCH [23]. In addition, the systemic pro-/anti-inflammatory deregulation found in FEP became more severe after a 1-year follow-up [24].

C-reactive protein (CRP) is a widely used biomarker of systemic inflammation, and higher CRP levels have been reported in SCH compared with HC patients [25–29], even in patients without antipsychotic treatment [30]. For homocysteine (Hcy), an intermediate amino acid containing a sulfhydryl radical that can act as an oxidant, the results are controversial. While some studies found higher levels of this oxidative stress biomarker in several subgroups of SCH [31–33] compared with HC patients [31,34], others did not [35,36].

A review of the literature suggests that there could be an overlap in peripheral immune-inflammatory mechanisms across severe mental disorders (SMD), what justify our research [37]. For example, Goldsmith et al. (2016) describe similarities in the pattern of cytokine alterations in SCH, bipolar disorder (BD), and major depressive disorder (MDD) during the acute (significant increases of IL-6, TNF-α, sIL-2R, and IL-1RA) and chronic (significant increases of IL-6, sIL-2R, and IL-1β) phases of illness that may suggest the existence of common underlying pathways for immune dysfunction [38]. Thus, it is necessary to evaluate markers of inflammation and immune activation across the whole psychosis continuum. In this sense, it was recently found that there is a strong increase in the levels of inflammatory activity in SCH and a relatively lesser increase in schizoaffective and affective disorders respectively [39]. In line with the proposed psychosis continuum model, the aim of our study was to identify the potential differential intra- and intercellular biochemical pathways controlling inflammatory response and their oxidative-nitrosative consequences in SCH, compared with BD and healthy controls (HC). An additional aim was to identify whether there are differential pathways of the psychopathological (positive, negative, and depressive), cognitive, and functional dimensions of SCH.

2. Methods

2.1. Study design

Cross-sectional, naturalistic study of a cohort of SCH patients in outpatient treatment at two mental health centers in Oviedo (Corredoria and Ería) in northern Spain, and their controls (BD patients and HC). The Clinical Research Ethics Committee of Hospital Universitario Central de Asturias in Oviedo approved the study protocol. All participants gave written informed consent prior to their enrollment.

2.2. Participants

Of the 325 participants recruited, a total of 305 individuals were included in the analysis after removing outliers. Of these, 123 were SCH patients (mean age 40.75, 67.5% males), 102 BD patients (mean age 48.37, 37.3% males), and 80 HC (mean age 35.81, 38.8% males). If an alfa error of 5% with a power of 90% is considered for the ANCOVA tests performed in this work, an effect size value of f = 0.2634 is obtained. This value, according to Cohen (1988) can be considered as a medium effect size and suitable for our research purposes [40].

Outpatients attending their regular appointments with their clinicians were offered to participate in the study and healthy controls were recruited by snowball sampling. There were statistically significant differences among the groups in age and gender ($F = 27.668$, $P < 0.0001$; $Chi^2 = 25.707$, $P < 0.0001$).

Inclusion criteria for SCH and BD were: (1) DSM-IV-TR diagnosis of SCH or BD; (2) age > 17 years; and (3) written informed consent. Inclusion criteria for HC: (1) no past or current mental disorder (DSM-IV-TR diagnosis) and (2) written informed consent. Exclusion criteria for patients and controls were: (1) no written informed consent; (2) physical comorbidity that could interfere with immune-inflammatory biomarkers (acute infection, fever, acute allergies, cancer, or autoimmune diseases) was determined by directly asking patients about them; (3) treatment with immunosuppressive drugs or vaccines within the 6 months prior to enrollment in the study, or treatment with anti-inflammatory drugs within the two days prior to blood collection.

2.3. Assessments

2.3.1. Psychometric instruments

Psychopathology in SCH patients was evaluated using the Spanish versions of the Clinical Global Impression (CGI) [41], which assesses the severity in global psychopathology, and the Positive and Negative Syndrome Scale (PANSS) [42], which measures the severity of positive, negative, and general psychopathology symptoms. In addition, the Negative Symptom Assessment-16 (NSA-16) [43] and the Hamilton Depression Rating Scale (HDRS) [44] were employed to assess the severity of negative and depressive symptoms, respectively. The Screen for Cognitive Impairment in Psychiatry (SCIP) [45] was used to assess cognition. Finally, to assess patient functioning, we used the Personal and Social Performance (PSP) [46].

2.3.2. Specimen collection and preparation

Venous blood samples (10 mL) were collected at 8:00 am after fasting overnight.

2.3.3. Biochemical analyses of PBMC samples

To perform all biochemical analyses, PBMC samples were first fractionated into cytosolic and nuclear extracts:

- preparation of cytosolic and nuclear extracts: to obtain a high purity nuclear fraction, practically without cytosolic contami-

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