Brain, Behavior, and Immunity 36 (2014) 111–117

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder

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ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form 17 October 2013
Accepted 17 October 2013
Available online 24 October 2013

Keywords:
Depression
Inflammation
Inflammasome
Oxidative stress
Amitriptyline
Antidepressants

ABSTRACT

Introduction: Major depressive disorder (MDD) is a very prevalent disease which pathogenic mechanism remains elusive. There are some hypotheses and pilot studies suggesting that cytokines may play an important role in MDD. In this respect, we have investigated the role of NLRP3 inflammasome complex in the maturation of caspase-1 and the processing of its substrates, IL-1β and IL-18, in blood cells from MDD patients.
Methods: Forty MDD patients were selected for this study, twenty without treatments and twenty treated with amitriptyline, a common tricyclic antidepressant. Blood samples from twenty healthy volunteers were included in the study. The inflammasome activation was studied by Western blot and real-time PCR of NLRP3 and caspase 1 and serum levels of IL-1β and 18.
Results: We observed increased gene expression of NLRP3 and caspase-1 in blood cells, and increased serum levels of IL-1β and IL-18 in non-treated patients. IL-1β and IL-18 correlated with Beck Depression Inventory (BDI) scores of MDD patients. Interestingly, amitriptyline treatment reduced NLRP3 and caspase-1 gene expression, and IL-1β and IL-18 serum levels. As it is well established that oxidative stress is associated with NLRP3 inflammasome activation, we next studied mitochondrial ROS and lipid peroxidation (LPO) levels in MDD patients. Increased levels of mitochondrial ROS and LPO were observed in MDD patients, however oxidative damage was higher in MDD patients treated with amitriptyline.
Conclusions: These findings provide new insight into the pathogenesis of MDD and the effects of amitriptyline treatment on NLRP3 inflammasome activation and IL-1β and IL-18 serum levels.

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1. Introduction

Major depressive disorder (MDD) is a severe and potentially debilitating psychiatric illness that is characterized by a significant change in mood accompanied by other symptoms such as low self-esteem, anhedonia, and disrupted sleeping, eating, and cognition. It has been estimated to be the second major illness in social and economic burden by 2020, exceeding all diseases except ischemic heart disease (Greden, 2001; Murray and Lopez, 1997). Despite the fact that it affects up to 10% of the general population worldwide, its pathogenic mechanism remains elusive (Kessler, 2012). Consequently, research is now aimed at characterizing its pathophysiology at the cellular and molecular level. There are some hypotheses and pilot studies suggesting that cytokines may play an important role in MDD (Gardner and Boles, 2011; Maes et al., 1993; Rawdin et al., 2012; Maes, 1995), associated with other alterations such as oxidative stress and mitochondrial dysfunction (Gardner et al., 2003; Gardner and Boles, 2011; Rawdin et al., 2012). A large body of evidence suggests that MDD is accompanied by activation of inflammatory pathways, reflected by an increased levels of inflammatory cytokines, such as Interleukin-1β (IL-1β), IL-6 and TNFα (Dowlati et al., 2010; Maes et al., 1993; Rawdin et al., 2012). Other small-scale studies also suggest antidepressant effects of anti-inflammatory medications (Muller et al., 2006; Nery et al., 2008), as well as anti-inflammatory effects of antidepressants (Hannestad et al., 2011; Abbasi et al., 2012). Interestingly, IL-1β is one of the two known cytokines, together with IL-18, activated by the inflammasome complex. The inflammasome is a protein complex that comprises an intracellular sensor, typically

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http://dx.doi.org/10.1016/j.bbi.2013.10.017

a Nod-like receptor (NLR), the precursor procaspase-1 and the adaptor ASC. Inflammasome activation leads to the maturation of caspase-1 and the processing of its substrates, IL-1β and IL-18 (Leemans et al., 2011). Of all the NLRs, NLRP3 is activated by the most diverse array of danger signals. Mitochondrial dysfunction and ROS have also been shown to be an important activator of inflammasome-mediated inflammation (Zhou et al., 2011). In agreement with these results, several mitochondrial alterations have been described in patients and animal models of depression, such as mitochondrial chain dysfunction, low levels of ATP or low levels of Coenzyme Q_{10} (CoQ_{10}) (Gardner et al., 2003; Maes et al., 2009; Moreno-Fernández et al., 2012; Rezin et al., 2008). However, it is not clear how mitochondrial alterations can be involved in the depressive process.

Recently, a new inflammasome hypothesis of depression and related comorbid systemic illnesses has been proposed by Iwata and co-workers (Iwata et al., 2013). According to this hypothesis, the inflammasome is a central mediator by which psychological and physical stressors could contribute to the development of depression (Iwata et al., 2013). The present study examined the hypothesis to determine whether NLRP3 inflammasome is activated in peripheral bloods mononuclear cells (PBMC) from MDD patients and the implication of mitochondrial oxidative stress. Furthermore, we studied the effect of amitriptyline, a tricyclic antidepressant, on NLRP3 inflammasome activation.

2. Material and methods

2.1. Ethical statements

Written informed consents and the approval of the ethical committee of University of Seville were obtained, according to the principles of the Declaration of Helsinki.

2.2. Patients

Twenty patients with major depression in first episode without treatment with a DSM-IV diagnosis of melancholic depression (DSM-IV code: F33) were collected from the Service of Psychiatry of Hospital Virgen Macarena, in Seville, Spain. The diagnostic was established by personal interview according to diagnostic criteria of DSM-IV. In parallel, we select twenty patients treated with amitriptyline in order to evaluate the effect of the antidepressant in inflammatory markers. All patients were also evaluated using the Beck Depression Inventory (BDI) (41.5 ± 8.3 is the mean of all depressed patients). Any suicide attempts were registered in all patients. All patients were recruited after a depressive episode. Patients not treated were included consecutively after diagnosis and before any antidepressant medication regimen was started. A few men were included in this study because of the low number of men affected by major depression in the Service of Psychiatry of Hospital Virgen Macarena at the time of the study. Patients on amitriptyline treatment were stable as confirmed by follow-up outpatient clinics. All patients followed chronic amitriptyline monotherapy treatment for a minimum of 10 months at doses considered clinically effective by their physicians. These doses were highly homogeneous (62.5 ± 12 mg/d). Patients without treatment had not taken medication before of the study and they were selected in the first episode. All patients were at least 18 years of age (age and sex in Table 1), and either they or their legal representatives gave informed consent to be included in the study. Blood samples from twenty healthy volunteers (males/5, females/15) were included in the study matching the age range, gender, ethnicity, and demographics of the recruited patients. The protocol has been carried out in accordance with the declaration of Helsinki and approved by the ethical committee of our institution.

Table 1
Age, sex, doses and time on treatment at the moment of peripheral venous blood isolation.

	Control	Non treated	Amitriptyline
No. patients	20	20	20
Age (yrs)	57 ± 3	54.4 ± 10.1	53.5 ± 9.8
Sex (female/male)	18/2	18/2	19/1
BMI (kg/m^2)	22.6 ± 5.1	23.3 ± 3.2	22.9 ± 3.9
Doses (mg/d)	-	-	62.5 ± 12
Months under treatment	-	-	12.1 ± 2.3

2.3. In vitro experiments

THP-1 cells were cultured at 37 °C in a 5% CO_2 atmosphere in RPMI-1640 medium supplemented with L-glutamine, an antibiotic/antimycotic solution (Sigma Chemical Co., St. Louis, MO, USA), and 10% fetal bovine serum. THP-1 cells were cultured with 5 mM ATP during 6 and 12 h with and without 0.5, 1 and 5 μM amitriptyline.

2.4. Immunoblotting

Western blotting was performed using standard methods. After protein transfer, the membrane was incubated with various primary antibodies diluted 1:1000, and then with the corresponding secondary antibody coupled to horseradish peroxidase at a 1:10000 dilution. Specific protein complexes were identified using the Immun Star HRP substrate kit (Biorad Laboratories Inc., Hercules, CA, USA). Anti-GAPDH monoclonal antibody from Calbiochem-Merck Chemicals Ltd. (Nottingham, UK). Anti-NLRP3 antibody from Adipogen (San Diego, USA).

2.5. Serum and blood mononuclear cells isolation

Coagulated and non-coagulated bloods were collected after 12-h fasting, between 8:00 and 10:00 AM from patients and healthy age- and sex-matched control subjects, centrifuged at 3800 g for 5 min, and serum was stored at −80 °C until testing. PBMC were purified from heparinized blood by isopycnic centrifugation using Histopaque-1119 and Histopaque-1077 (Sigma Chemical Co., St. Louis, MO, USA).

2.6. IL-1β and IL-18 levels

IL-1β and IL-18 levels in serum or culture mediums were assayed in duplicates by commercial ELISA kits (MyBioSource, Inc., CA, USA).

2.7. Real-time quantitative PCR

The expression of NLRP3 and caspase 1 gene was analyzed by SYBR Green quantitative PCR using mRNA extracts of PBMC from patients and controls. Total cellular RNA was purified from PBMC using the Trisure method (Bioline, London, UK), according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically. In order to avoid genomic DNA contamination, one microgram of total RNA from each sample was incubated in gDNA wipeout buffer (Quantitect Reverse Transcription Kit, Qiagen. Hilden, Germany) at 42 °C for 5 min. RNA samples were subsequently retrotranscribed to cDNA using QuantiTect Reverse Transcription Kit (Qiagen. Hilden, Germany). The thermal cycling conditions used were: denaturation at 95 °C for 20 s,

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