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Escitalopram or novel herbal treatments differentially alter cytokine and behavioral responses to immune challenge

Ronit Avitsur[a,*], Shalva Paley[a,c], Motty Franko[a], Nizan Wolff[a], Nir Eyal[a], Ravid Doron[a,b]

[a] School of Behavioral Sciences, The Academic College of Tel Aviv-Yaffo, Tel Aviv, Israel
[b] Department of Education and Psychology, The Open University of Israel, Israel
[c] Department of Psychology, Tel Aviv University, Israel

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ABSTRACT

Studies suggest that inflammation is involved in the pathophysiology of depression. The present study examined the effects of the commonly used antidepressant escitalopram, in comparison with a novel herbal treatment (NHT) consisted of *Crataegus pinnatifida*, *Triticum aestivum*, *Lilium brownii* and *Fructus Ziziphus jujuba*, on cytokine and behavioral responses to an immune challenge. Escitalopram augmented lipopolysaccharide-induced tumor necrosis factor (TNF)-α peripheral secretion and induced a faster kinetics of interleukin-1β secretion, while marginally reducing sickness behavior. NHT, on the other hand, completely abolished lipopolysaccharide-induced interleukin-1β and TNFα peripheral secretion and diminished sickness behavior. These findings may have implications for the treatment of depressive symptoms associated with immune activation.

1. Introduction

Major depression disorder (MDD) is a frequent and disabling psychiatric disorder. Comprehensive understanding of the underlying disease processes in MDD is currently lacking. Studies have suggested that an imbalance in monoaminergic neurotransmission is at the core of the pathophysiology (Massart et al., 2012; Nestler et al., 2002) and interactions between genetic risk factors and life adversities increase susceptibility to MDD (Caspi et al., 2003).

Studies further suggested that inflammation plays a role in the pathophysiology of depression in subgroups of patients (Dantzer et al., 2008; Maes, 1995; Miller et al., 2009; Raison et al., 2010). Accumulating evidence indicate that depressed patients who are otherwise healthy exhibited increased levels of pro-inflammatory cytokines and other inflammatory biomarkers both in the periphery and in the cerebrospinal fluid compared to non-depressed individuals (Miller et al., 2009). A meta-analysis of cytokine levels in patients with major depression identified significantly higher levels of Major depression disorder (MDD)-α and interleukin (IL)-6 compared to controls (Dowlati et al., 2010). Further evidence indicated that inflammation is not generally present in MDD patients, but probably restricted to specific subgroups of depressed persons (Haroon et al., 2012; Raison and Miller, 2011).

A large number of MDD patients use antidepressant medications, particularly selective serotonin reuptake inhibitors (SSRIs). Several studies indicated that SSRI antidepressants played a modulatory role on immune function in MDD patients. Reports indicated that SSRI antidepressant therapy was associated with a normalization of plasma proinflammatory cytokines levels (Hannestad et al., 2011) and patients with treatment-resistant depression had elevated plasma cytokine levels compared to patients in remission (O'Brien et al., 2007; Yoshimura et al., 2009). Along the same lines, escitalopram, the most selective of the SSRIs (Owens and Rosenbaum, 2002), increased the levels of interleukin (IL)-1 receptor antagonist and IL-2 in MDD patients (Ho et al., 2015). Moreover, escitalopram contributed to a shift toward T helper 2 responses and was associated with an increase in modulators of innate immunity, resulting in a decrease in innate and adaptive of immune function (Ho et al., 2015). An additional study reported that changes in serum levels of soluble IL-2 receptor were different for responders and non-responders to escitalopram treatment in MDD patients (Eller et al., 2008). However, Haastrup et al. (2012) did not find an effect of escitalopram on cytokine levels in patients with clinical depression.

In support of the cytokine hypothesis of depression are the reports indicating that administration of bacterial endotoxin lipopolysaccharide (LPS) elevated proinflammatory cytokine levels. LPS also induced symptoms of sickness behavior that are similar to the core

Abbreviations: BDNF, Brain-derived neurotrophic factor; IL, Interleukin; LPS, Lipopolysaccharide; MDD, Major depression disorder; NHT, Novel herbal treatment; SSRI, Selective serotonin reuptake inhibitor; VEH, Vehicle control

* Corresponding author at: School of Behavioral Sciences, The Academic College of Tel Aviv-Yaffo, 14 Rabenu Yerucham St., PO Box 8401, Tel Aviv 68114, Israel.
E-mail address: avitsur@mta.ac.il (R. Avitsur).

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symptoms of depression, although differ in their temporal kinetics (Dantzer et al., 2008; O'Connor et al., 2009). Moreover, SSRIs oftentimes contradicted the effects of endotoxin. A single exposure to SSRI antidepressant (fluoxetine or paroxetine) prevented many aspects of sickness behavior and blunted proinflammatory cytokine response to endotoxin administration (Dong et al., 2016; Ohgi et al., 2013; Roumestan et al., 2007; Yao et al., 2015). Chronic treatment with fluoxetine attenuated LPS-induced anorexia and body weight loss (Yirmiya et al., 2001), however chronic treatment with paroxetine or venlafaxine failed to alter LPS-induced sickness behavior (Shen et al., 1999).

Recent studies from our laboratory introduced a novel herbal treatment (NHT) that may be helpful in the treatment of mood disorders. NHT is prepared from four herbal components: *Crataegus pinnatifida*, *Triticum aestivu*, *Lilium brownii* and *Fructus Ziziphus jujuba*, as a modification of a classical Chinese formula used in the treatment of mental disorders since the 2nd century A.D (Scheid et al., 2009). In mice, the NHT mixture reduced anxiety- and depressive-like behaviors and suppressed corticosterone response to chronic mild stress. NHT also increased brain-derived neurotrophic factor (BDNF) levels in the hippocampus and prefrontal cortex and increased hypothalamic levels of the serotonin transporter (Doron et al., 2014a, 2014b).

The effects of NHT on anxiety- and depressive-like behaviors were similar to those of chronic treatment with the SSRI antidepressant escitalopram in chronically stressed mice (Doron et al., 2014b). Based on these findings, it was suggested that NHT may be beneficial in treating anxiety and depression (Doron et al., 2014a, 2014b). Since depression was associated with changes in immune activity, the present study sought to examine the effects of NHT, in comparison with the effects of escitalopram, on proinflammatory cytokine secretion and sickness behavior following an immune challenge.

2. Methods

2.1. Animals

Subjects were male ICR (CD1) mice (Harlan Laboratories, Israel), aged 2–4 months. Subject was housed at the Academic College of Tel Aviv-Yaffo Animal facility. Room temperature was 22 ± 2 °C. All subjects were given free access to food and water and were maintained on a reversed 12-hour light/dark cycle (lights on at 7:00 PM). Subjects were group-housed (4–5/cage) unless otherwise noted. All experimental procedures were performed during the dark phase under red light illumination. Animal-care procedures were approved by the Israel National Committee of Animal Care and Use. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. The animals receiving the NHT did not experience physical or behavioral adverse effects.

2.2. Drugs

2.2.1. Novel herbal treatment (NHT)

Crataegus pinnatifida, *Triticum aestivum*, *Lilium brownii* and *Fructus Ziziphus jujuba* were purchased as freeze-dried granules from KPC Products, Inc. (Irvine, CA, USA). The NHT solution was prepared by dissolving the four compounds (together) in saline containing 1% DMSO and administered at a dose of 30 mg/kg/day, i.p. Previous studies demonstrated that this dose of NHT reduced anxiety- and depressive-like behavior and increased BDNF levels in the hippocampus and mice (Doron et al., 2014a, 2014b).

2.2.2. Escitalopram

Escitalopram was administered at a dose of 15 mg/kg/day, i.p., diluted in saline containing 1% DMSO. Escitalopram is one of the most efficient and commonly prescribed antidepressant drug (Cipriani et al., 2012), and was therefore chosen as a positive control for the antidepressant effects of NHT. Previous studies from our laboratory showed that the effects of this dose of escitalopram on anxiety- and depressive-like behavior and on hippocampal BDNF levels was comparable to that of the NHT dose used in the current study (Doron et al., 2014a, 2014b). Escitalopram was donated by TEVA Ltd. (Israel).

2.2.3. Lipopolysaccharide (LPS)

LPS (from *Escherichia coli* 0111:B4, Sigma) was diluted in PBS containing 1% DMSO and administered at a dose of 5 μg/mouse, i.p. The dose was chosen based on previous studies using similar procedures (Avitsur and Sheridan, 2009; Avitsur et al., 2013).

2.2.4. Vehicle control treatment

Control mice for the NHT and escitalopram chronic treatments, as well as for the LPS exposure, were administered with a vehicle containing sterile saline and 1% DMSO, ip.

2.3. Cytokines assessment

Plasma cytokine levels were assayed using Quantikine mouse cytokine immunoassay solid phase ELISA Kit (R & D Systems, Minneapolis, USA). Assay values were (according to the manufacturer): **Interleukin-1 beta (IL-1β)**: Precision: intra-assay 1.5–4.4%, inter-assay 2.8–6.1% according to level. Specificity: no significant cross-reactivity was observed excluding human IL-1β (1%) rat IL-1β (4%); range of detection 7.8–500 pg/ml. Values of IL-1β content in the hippocampus are presented after normalization to total protein levels using a standard Bradford assay. **Tumor Necrosis Factor alpha (TNFα)**: Precision: intra-assay 4.3–9%, inter-assay 6.1–9.2% according to level. Specificity: cross-reactivity with rat TNFα – 78%, all others – negligible; range of detection 23.4–1500 pg/ml.

2.4. Procedure

2.4.1. Experiment 1: effect of herbal treatment or escitalopram on LPS-induced proinflammatory cytokine secretion

Subjects were randomly assigned to three treatment groups: vehicle control (VEH), NHT and escitalopram. Animals were administered with the drugs daily for three weeks. Injections were performed between 9:00 and 11:00 AM during the dark phase of the diurnal cycle. Following chronic drug treatment, animals from each group (VEH/NHT/escitalopram) were injected with either VEH or LPS. Blood was collected by facial vein puncture (Avitsur et al., 2013; Golde et al., 2005) 2, 4, 6, or 24 h following VEH/LPS injections into EDTA coated tubes. Plasma was separated and stored at − 20 °C until assayed for levels of IL-1β, or TNFα. Separate groups of mice were used to assess levels of each cytokine at each different time point. Each experimental group (same chronic and acute manipulations, same time of blood collection) included 4–8 subjects.

2.4.2. Experiment 2: effect of herbal treatment or escitalopram on LPS-induced sickness behavior

To assess sickness behavior, changes in body weight, food and sucrose consumption following LPS administration were examined. Subjects were randomly assigned to three treatment groups: VEH, NHT and escitalopram. Animals were administered with the drugs daily for three weeks. Injections were performed between 9:00 and 11:00 AM during the dark phase of the diurnal cycle. Subjects were individually housed at least 48 h before the beginning of sickness behavior measurements. Water bottles in each cage were replaced with a bottle of sucrose solution (2% in dH_2O, Sigma) for a period of 24 h, followed by a period of at least 24 h with no exposure to sucrose (water only). For baseline measurements, animals were weighed and supplied with weighed food and two drinking bottles: water and sucrose. Body weight, food and drinking bottles were weighed again 2, 4, 6, and 24 h