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FLUOXETINE AND S-CITALOPRAM INHIBIT M1 ACTIVATION AND PROMOTE M2 ACTIVATION OF MICROGLIA *IN VITRO*

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Abstract—Increasing evidence has suggested that microglia dysfunction plays an important role in the pathogenesis of depression. Both classical activation (M1 activation) and alternative activation (M2 activation) may be involved in the process. M1-activated microglia secrete various pro-inflammatory cytokines and neurotoxic mediators, which may contribute to the development of depression, while M2-activated microglia promote tissue reconstruction by releasing anti-inflammatory cytokines involved in the process of depression. Selective serotonin reuptake inhibitors (SSRIs) are first-line treatments for depression, and their effects on immune system modulation have recently gained attention. Several studies have suggested that SSRIs affect the M1 activation of microglia, but results have varied. In addition, little is known about the effect of SSRIs on M2 activation in depression. The aim of this study was to investigate the effects of fluoxetine and S-citalopram, two widely used SSRIs in clinical, on both the M1 and M2 activation of microglia (the murine BV2 cell line and mouse primary microglia cell). The indexes of activation were measured by real-time polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) and Western blot. The present results showed that both fluoxetine and S-citalopram significantly down-regulated the indexes of M1 activation and up-regulated the M2 activation indexes on mRNA and protein levels either in cell line or primary cells. Taken together, the results suggested that fluoxetine and S-citalopram modulated the immune system by inhibiting M1 activation and by improving M2 activation of microglia and that the immune system modulation may partially mediate the therapeutic effects of antidepressant drugs-SSRIs.
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Abbreviations: CD86, cluster of differentiation 86; DMEM, Dulbecco' s modified Eagle medium; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; IFN-γ, interferon-γ; IL-6, interleukin-6; LPS, lipopolysaccharide; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; NO, nitric oxide; SOCS3, suppressor of cytokine signaling 3; SSRIs, selective serotonin reuptake inhibitors; STAT, signal transducers and activators of transcription; TNF-α, tumor necrosis factor-α.

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Key words: depression, microglia, fluoxetine, S-citalopram.

INTRODUCTION

The relationship of inflammation with depression has been supported by some well-known observations. For examples, major depressive disorder (MDD) is associated with raised inflammatory markers (Dahl and et al., 2014) and inflammatory medical illnesses (Lo and et al., 2010). In addition, patients treated with cytokines for various illnesses are at an increased risk of developing depression (Wichers and Maes, 2002). Even though the association between inflammation and depression is not consistently significant in all studies or for all cytokines, the positive results of meta-analysis gave convincing evidence for the relationship, and the heterogeneity of different studies may arise from the differences of age, gender, race, symptom severity and the phase of illness (Dowlati and et al., 2010). Based on these observations, the hypothesis has been posited, in which inflammatory processes might contribute to the development of depression, and numerous studies have attempted to explain how inflammatory processes cause changes in brain structure and function related to depression (Capuron and Miller, 2011; Raison and Miller, 2011). Increasing evidence suggested that inflammation can reduce neurogenesis (Barrientos and et al., 2003; Iosif and et al., 2006; Ben and et al., 2008), motivate the activity of hypothalamic–pituitary–adrenal (HPA) axis Miller et al., 1999; Hu et al., 2009 and inhibit the function of serotonin system (Yang and et al., 2004; Fujigaki and et al., 2006; Zhu et al., 2006), all of which are believed to play key roles in the development of depression. The above evidences have implicated inflammation involves in the etiology of depression and have formed the foundation of the neuro-inflammation theory of depression.

The dysfunction of microglia in depression is now gaining much more attention, as the microglia cell is generally considered as the most important immune cell in the central nervous system. In healthy brains, the microglia are 'resting' and play the role of immune supervision (Nimmerjahn et al., 2005). However, in pathological conditions, microglia can be activated by various stimulations (Aloisi, 2001), and different stimulations induce the activation of microglia into a 'classical (M1)' or 'alternative (M2)' activated states (Colton and Wilcock, 2010). The M1-activated cells undergo rapid proliferation, express activation markers [such as cluster of

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differentiation 86 (CD86)] and secrete various pro-inflammatory cytokines [such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin 1-β (IL-1β)] and cytotoxic factors [such as nitric oxide (NO) and reactive oxygen–nitrogen species] (Aloisi, 2001). Microglia cells in this state generally act in tissue defense and promote the destruction of pathogens (Kettenmann and et al., 2011). However, the microglia cells can induce inflammo-toxicity of the healthy tissue at the same time (Colton and Wilcock, 2010). Hence the M2-activated microglia cells are key aspects in keeping the homeostasis in the CNS by secreting anti-inflammatory cytokines [for example, IL-10 and transforming growth factor-β (TGF-β)] to down-regulate the pro-inflammatory process and initiate tissue reconstruction (Kettenmann and et al., 2011). The balance between M1/M2 activation is extremely important in keeping healthy and resisting diseases. It has been suggested that excessive M1 activation and deficiency of M2 activation is of great significance in the etiology and pathogenesis of depression. In addition, anti-inflammatory medications may be beneficial in the treatment process (Eyre et al., 2014).

Selective serotonin reuptake inhibitors (SSRIs) have been a first-line choice for the treatment of depression for several decades, and the SSRIs act, at least in part, by increasing monoamine transmission. However, in recent decades, the antidepressants have been proven to modulate the inflammation process (Roumestan and et al., 2007; Hannestad et al., 2011). Clinical studies showed that the elevated serum levels of pro-inflammatory cytokines in depressed patients are often turned to normalization after successful treatment with antidepressants (Hannestad et al., 2011). *In vitro* studies further support the finding by showing that SSRIs can inhibit the activation of peripheral immune cells from either human or rodent (Roumestan and et al., 2007). Although the SSRIs affect the immune cell in the peripheral nervous system, it is not reasonable to conclude that SSRIs modulate the inflammation process in the CNS and that the therapeutic effects may be related to inflammation. Recently, several studies have shed light on this issue by focusing on the effects of SSRIs on microglia activation. However, the results of such studies have been variable and even contradictory (Ha et al., 2006; Hashioka and et al., 2007; Lim and et al., 2009; Horikawa and et al., 2010; Liu and et al., 2011; Lee and et al., 2011; Tynan and et al., 2012; Du et al., 2014). Furthermore, all the studies only focused on the M1 activation of microglia affected by SSRIs, and no study to date has investigated the effect of SSRIs on the M2 activation of microglia.

Accordingly, in the current study, we sought to investigate the effect of fluoxetine and S-citalopram, two widely used SSRIs, on both the M1 and M2 activation of microglia via the research of the murine BV2 cell line and primary microglia cell *in vitro*. The present study had investigated whether fluoxetine and S-citalopram reduce inflammation indexes of M1 activation and increase the indexes of M2 activation, and whether the effect was concentration-dependent. In summary, the study may provide new evidence concerning the pharmacological mechanism of SSRIs, especially their promotion of M2 activation of microglia, which may contribute to understanding the pathogenesis and developing new treatment options for depression.

EXPERIMENTAL PROCEDURES

Reagents

Fluoxetine, lipopolysaccharide (LPS), recombinant interferon-γ (IFN-γ) and IL-4 were all purchased from Sigma–Aldrich (USA). S-citalopram was purchased from Lundbeck (Denmark). Fluoxetine and S-citalopram were initially dissolved in phosphate-buffered saline (PBS, 150 mM NaCl, 5 mM phosphate, pH 7.4). LPS, recombinant IFN-γ and IL-4 were initially dissolved in sterile distilled–deionized water. In all cases, subsequent dilutions to working concentrations were made using cell culture media.

BV2 microglial cell culture

BV2 cells, which were purchased from Cell Bank of Chinese Academy of Sciences, Shanghai, China, were maintained in Dulbecco' s modified Eagle medium (DMEM, Invitrogen, USA) with 10% fetal bovine serum (FBS, Invitrogen, USA) in a 5% CO_2 incubator. Plated cells were grown in DMEM with 10% FBS overnight. In all experiments, cells were treated with LPS (200 ng/ml) and INF-γ (20 ng/ml) or IL-4 (10 ng/ml) in the absence or presence of the indicated concentrations of fluoxetine or S-citalopram (20 or 60 μM) in serum-free DMEM.

Primary microglial cell culture

Primary microglia cells were prepared from the whole brains of 1–2-day-old SD rat. Briefly, the whole brain were chopped and dissociated by mechanical disruption using a nylon mesh. The cells were seeded in poly-D-lysine-coated flasks. After *in vitro* culture for 14 days, microglia cells were isolated from mixed glia cultures by mild trypsinization. The prepared primary microglia cultures were more than 95% pure, as determined by CD11b immunocytochemical staining (data not shown). Cells were cultured for 24 h before drug treatment. The experimental protocol was carried out in accordance with the European Communities Council Directive of 24 November 1986 and we spared no efforts to minimize the number of animals used and their suffering.

Cell viability assay

Cell viability was determined by the tetrazolium salt 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma–Aldrich, USA) assay. Microglia cells were plated into 96-well culture plates at a density of 5×10^4 cells/ml with 200-ml culture medium per well. Following treatment with different concentrations (1, 10, 20, 40, 60, 80, and 100 μM) of fluoxetine or S-citalopram for 24 h, 5 mg/ml MTT solution was added to each well and incubated at 37 °C for 4 h. The medium was aspirated and 200-ml dimethyl sulfoxide was added. The absorbance value was measured using

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