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Suppression of pro-inflammatory cytokine expression and lack of anti-depressant-like effect of fluoxetine in lipopolysaccharide-treated old female mice

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ABSTRACT

Some antidepressants show a significantly lower efficacy in elderly patients, particularly in women. Previous studies have shown that antidepressants administered to young animals reduced depression-like symptoms induced by lipopolysaccharide (LPS). The aim of this study was to find out whether the antidepressant and anti-inflammatory properties of fluoxetine (FLU) can be observed also in old female C57BL/6J mice. A depression-like state was evoked by the administration of LPS (100 μg/kg for 4 consecutive days) which was followed by reduction of sucrose preference (anhedonia) and enhancement of immobility-time in the forced swim test (FST). Animals, which received FLU (10 mg/kg, 11 days) exhibited a decreased LPS-induced expression of some inflammatory cytokines in the hippocampus and spleen but this effect was not accompanied by beneficial changes in animals' behavior. Despite the lack of antidepressant-properties of FLU in this model, our studies have proven significant profound anti-inflammatory properties of chronic FLU treatment which may suggest its suitability for fending off inflammatory processes in the elderly.

1. Introduction

Depression is widespread among old people, with a prevalence ranging from 22 to 46% in people over 65 years old [1]. The number of depressed people will probably increase from 52 million in 2010 to 1.5 billion in 2050 because of expected increase in aged population (WHO, 2011). The risk of development of depression is higher in old people who need primary care services or are inmates of nursing homes [2,3]. In addition, results of clinical studies constantly pointed to gender differences among patients with depression, with women outnumbering men at a rate of 5:1, in “perimenopause age” [4–6].

Depression in elderly people more often is comorbid with diseases characterized by activation of pro-inflammatory components [7]. Depression is particularly prevailing in people with chronic illnesses, such as ischemic heart disease, stroke, cancer, chronic lung disease, arthritis, Alzheimer's disease and Parkinson's diseases [8]. Moreover, depressed older patients are more likely to develop additional diseases and more severe symptomatology than young adults [9,10], which might contribute to the high rates of suicide among the elderly [11]. Risk factors for developing depression increase with age because the homeostatic reserves of the organism to overcome biological challenges are lower [12–14].

The effectiveness of antidepressants in older individuals has not been systematically assessed. Some reports suggest that older patients are less responsive to antidepressants than young adults [15,16], but this view has not been fully supported. Clinical studies on the therapeutic efficacy of selective serotonin reuptake inhibitors (SSRI) in older population brought highly variable results. Most studies showed that SSRI were superior to placebo in elderly patients with major depressive disorder (MDD) but they were not more efficient than placebo in elderly patients suffering from minor depression or dysthymia [17]. On the other hand, it was shown that fluoxetine had significantly lower efficacy than placebo in elderly patients with MDD [18]. Moreover, SSRI were found to be efficacious in patients 55 to 65 years old but were less active in the subset of studies using age thresholds of 65 years or older [19]. These conditions underscore the

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relevance of clinical and experimental studies into the effectiveness of antidepressant drugs in aged population.

Lipopolysaccharide (LPS) is a highly conserved cell wall component of Gram-negative bacteria that is recognized by the immune system of higher vertebrates as a pathogen-associated molecular pattern (PAMP). Inflammatory response to LPS is associated with activation of toll-like receptors (TLRs). TLR4 is considered to be the major LPS signaling receptor. The formation of an LPS-TLR4/MD-2 complex and subsequent recruitment of myeloid differentiation primary-response gene 88 (MyD88), activating downstream nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) pathways leading to up-regulate expression of the inflammatory cytokines: interleukin 1β (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor α (TNF-α), among other genes [20]. These released cytokines then relay signal to the central nervous system (CNS) macrophages and microglia to produce the same cytokines, targeting neuronal substrates and eliciting sickness behavior [21].

Several biological mechanisms with a possible role in MDD pathophysiology have been considered and accumulating evidence suggests that inflammation plays an important role both in the etiology and progression of this disease. Meta-analysis showed higher blood levels of pro-inflammatory cytokines, such as TNF-α and IL-6, in drug-free depressed patients compared with healthy controls [22], whereas a postmortem brain study showed elevated gene expression of pro-inflammatory cytokines in the frontal cortex of people with a history of MDD [23]. Moreover, therapeutic administration of interferon (IFN)-α in patients with hepatitis-C, increased pro-inflammatory cytokine levels in serum, leading to depressive symptoms [24,25], which could be reversed by antidepressant drugs thus reducing depressive symptoms.

In animal studies, peripheral administration of bacterial endotoxin LPS induces depressive-like behavior [26,27] and antidepressant efficacy of SSRI has been verified in this animal model of depression in adult but not senescent animals. It is still uncertain whether the immune effects of antidepressants are necessary for their therapeutic efficacy. Therefore, in the present study, we examined the effect of fluoxetine on behavioral changes and pro- and anti-inflammatory cytokine mRNA expression in the hippocampus and spleen in senescent 22–23 months old C57BL/6J females with LPS-induced "depression-like" state. First, we evaluated the effect of fluoxetine on LPS-induced depression-like behavior in mice using the sucrose preference test and the forced swim test (FST). Second, we examined the anti-inflammatory potential of fluoxetine in the LPS-induced model, by assessing the changes in the mRNA levels coding for IL-1β, TNF-α, IL-6 and IL-10 in the hippocampus and spleen.

2. Materials and methods

2.1. Mice

Inbred female C57BL/6J mice from the animal facility at the Department of Experimental Neuroendocrinology of the Institute of Pharmacology in Krakow, Poland were used. Female mice were group-housed (47 females kept in 12 cages) at a constant temperature of 22 °C by at least 12 months before experiment. The mice received pelleted food ad libitum. The experiment was performed when the mice were 22–23 months old.

All procedures were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), and the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were approved by the 2nd Local Bioethics Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. All efforts were made to minimize the number of animals and their suffering.

2.2. Behavioral measurements, fluoxetine and LPS administration

To assess the sucrose preference, female C57BL/6J mice were trained for two weeks to drink a sucrose solution. They were given a two-bottle choice between a sucrose solution (1% w/v) and water [26]. The positions of two bottles were switched daily to reduce confounds caused by side preference. Both sucrose and water solutions were available ad libitum. Using this experimental design, we confirmed our former observation that the animals showed a preference for the sucrose solution and drank little water [26]. While some authors recommend a 14-hour food and water deprivation before "one bottle sucrose solution test", we studied, according to Shen et al. [28], liquid consumption behavior under more natural conditions, i.e. when the need for food and water deprivation was obviated. Water and sucrose solution intakes were measured by weighing each bottle. After two-week training, the mice were divided into two matched groups and treated intraperitoneally with: fluoxetine (FLU, 10 mg/kg, Lilly Laboratories, USA) or saline (Veh, 1 mg/ml)-treated for 11 days. After 7 days of FLU or Veh administration mice were further subdivided into two subgroups which for the subsequent four days received daily intraperitoneal injection of *Escherichia coli* LPS (Serotype 0127:B8, Sigma-Aldrich Chemical Co., St. Louis, USA) in a dose of 100 μg/kg or Veh. Fluoxetine; injections were performed 1 h before LPS injection. Fresh solutions of FLU and LPS were prepared by dissolving compounds in sterile endotoxin-free isotonic saline. We employed a model of repeated LPS injections to induce depression-like behavior and inflammatory stress in mice. Bottles with water and 1% sucrose solution were weighed three times a week during the two-week training to drink sweet solution and daily during the LPS and/or FLU injection period (24 h after each LPS and/or FLU dose). The preference for sucrose was assessed as the ratio of sucrose consumption/total fluid consumption. In the statistical analyses, we used the main index, defined as the amount of liquid consumed per period between two sequential bottle weightings divided by the appropriate number of days (i.e. one, two or three) and by the number of animals per cage. Thus, the main index is an average amount of sweet and tap solution drank per 24 h and per mice.

Dose of FLU was selected based on our previous reports dealing with the antidepressant effect of repeated FLU treatment in C57BL/6J mice [26,29,30].

The forced swim-test was performed 22 h after the third LPS dose and/or 23 h after 10th Veh or FLU injection. The mice were placed in glass beakers, filled with water (21-23 °C) up to a height of 30 cm. The total duration of floating (immobility time), swimming, and climbing period was scored by the same observer during the last 4 min of a 6-min test. Water in the beakers was changed after each mouse.

2.3. Proliferation assay

The animals were sacrificed by decapitation at two time points: 2 and 24 h after the last fourth dose of LPS or 3 and 25 h after the last saline or fluoxetine injection. The proliferative activity of splenocytes was described in our earlier paper [31]. Briefly, the spleens of rats were aseptically dissected and gently crushed in a glass homogenizer. Cells were suspended in RPMI-1640 medium and were centrifuged at 500 × *g* for 5 min. The cell pellets were re-suspended in the same medium supplemented with antibiotics and 10% fetal calf serum. Spleen cells (4×10^6 cells/ml) were stimulated by concanavalin A (Con A; 0.6 μg/ml and 1.25 μg/ml) and by LPS (5 μg/ml) and were incubated in 96-well plates at a final volume of 0.2 ml for 72 h. Cell proliferation was determined by adding 0.5 μCi of [^{3}H]-thymidine per well (ICN, USA; Spa 6.7 Ci/mmol) at 16 h before the end of the incubation.

2.4. mRNA extraction, quantification and Real-Time PCR

The expression of selected cytokines (IL-1β, TNF-α, IL-6 and IL-10) in