IL-10 after chronic stress and probiotic use in adolescent Sprague-Dawley rats.

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Abstract

Exposure to early life stress (ELS) increases vulnerability to neuropsychiatric disorders such as depression. Anti-inflammatory cytokine IL-10 has been reduced in people with depression. Treatment with the probiotic *L. rhamnosus* has led to decreased depressive behaviors, while another probiotic *B. infantis* was found to increase IL-10 levels. The current study aims to determine if treatment of *L. rhamnosus* can counteract the reduction in IL-10 levels seen in adolescent depression. A recent model of female adolescent depression will be used, to assess its applicability in males. All animals will be divided into eight groups of nine, powered to identify sex differences, with a 2(male, female) X 2(no-stress, NMD*CMS) X 2(saline, probiotic) study design. The current study will produce ELS in Sprague-Dawley rats through neonatal maternal deprivation (NMD) followed by chronic mild stress (CMS). Saline or *L. rhamnosus* will be orally gavaged for all animals. Following decapitation, the hypothalamus, prefrontal cortex, and blood plasma will be collected, and standard assays will be used to assess anti-inflammatory IL-10 cytokine concentrations. Results are expected to demonstrate a reduced IL-10 concentration for the NMD*CMS condition, an effect moderated in the *L. rhamnosus* groups. The significance of this study is to further determine the variation of IL-10 after adolescent depression, and the efficacy of *L. rhamnosus* to moderate these effects for each sex.

Keywords: IL-10, early life stress, neonatal maternal deprivation, probiotics, *L. rhamnosus*,anti-inflammatory, chronic mild stress, sex difference

Introduction

Previous research has shown that pro-inflammatory cytokines are linked to depression. These include interleukins IL-6 and IL-1B, as well as tumor necrosis factor-a (TNFa). Concentrations of IL-1B, IL-6, and TNFa are increased in individuals who experienced adversity early in life, as compared to those without this experience (Müller et al, 2019). Additionally, inflammatory states has been assessed as biomarkers to predict the development of PTSD (Michopoulos et al, 2019). Studies linking cytokines and early life stress have focused in large part on pro-inflammatory effects. Anti-inflammatory cytokine IL-10 has been seen to have reduced concentrations in plasma for people who endured early life stress (ELS) (Müller et al, 2019). Genty et al (2017, 2018) have studied the relationship between ELS and inflammation as well as depression and resilience to psychoses and stress, seen in reduced stress-induced corticosterone levels. Anti-inflammatory cytokine IL-10 was found to play a greater role in depression than in PTSD (Genty et al, 2018). Gouin et al (2017) found that with the use of external resilience resources, levels of pro-inflammatory cytokine IL-6 were reduced in participants who had undergone ELS.

Probiotics have begun to be used as researchers examine the gut-brain connection. Among these is the lactic acid bacteria *Lactobacillus rhamnosus (JB-1)* (*L. rhamnosus*). A 2011 study showed that treatment with *L. rhamnosus* led to a reduction in stress-induced corticosterone (a marker of resilience), a decrease immobility duration during Forced Swim Testing (indicating reduced depressive-like behaviors), and a reduction in anxious behavior during the Elevated Plus Maze (Bravo et al, 2011). Importantly, probiotics have been studied in relation to cytokine levels as an indication of inflammation in patients with Irritable Bowel Syndrome (IBS). A 2005 study showed that patients with IBS had reduced IL-10 levels. After

treatment with *B. infantis*, the ratio of pro- and anti-inflammatory cytokine levels were normalized (O'Mahony et al, 2005). These prior studies demonstrate possible counteracting effects of probiotics on IL-10 following a stress model.

Goals/Hypothesis

The goal of the proposed experiment is to determine whether there is a sex difference in efficacy of the paired neonatal maternal deprivation/ chronic mild stress procedure in the reduction of IL-10 concentration in the hippocampus, prefrontal cortex and plasma, as well as in the response to *L. rhamnosus* under these conditions. It focuses on the under-studied pleiotropic cytokine IL-10 and addresses the plausibility of probiotics as treatment for depression, with a hope to define a path toward treatment of the inflammatory effects of adolescent depression.

If chronic stress reduces IL-10 and L. rhamnosus increases IL-10, treatment with L. rhamnosus should counteract the immune effects of adolescent depression in Sprague-Dawley rats. Male rats in the stress condition are expected to have higher IL-10 levels than females in the same condition, as females experience higher incidence of depression.

Methodology

Overview

Neonatal maternal deprivation followed by chronic mild stress was found to be an effective model for depression in Sprague-Dawley adolescent female rats (Ye et al, 2019), in which pro-inflammatory cytokines IL-1B, IL-6, and TNFa were found to increase.

Following a power analysis, it was determined that 9 rats are needed per group in order to analyze sex differences. A total of 72 Sprague-Dawley rats will be obtained. Rats will be grouped into eight groups composed of the following conditions per sex: no-stress*saline, no-stress*probiotic, NMD*CMS*saline, NMD*CMS*probiotic.

All neonatal rats will be kept under standard housing conditions (12/12 h light/dark cycle, lights on at 7:00 AM, controlled temperature and humidity). *L. rhamnosus* or saline will be administered by oral gavage from PND 22 to 42.

Stress Simulation

Neonatal maternal deprivation. (adapted from Ye et al, 2019)

From PND 2 to PND 21, pups in the stress conditions will be removed from their home cages and placed on electric heating pads of the same temperature as the animal room. NMD lasted for 3 h each day and animals will be returned to the home cage after changing the sawdust. At post-natal day (PND) 21, all rats will be weaned, then pair-housed from PND 22 to PND 42.

Chronic Mild Stress. (adapted from Ye et al, 2019)

NMD*CMS rats will be subjected to one mild stressor each day, beginning on PND 22. The procedure will last 3 weeks. The first two weeks will be a cycle involving one of the following stressors each day: food deprivation for 8 h, water deprivation for 8 h, inversion of day/night cycle, forced swimming for 3 min, 45° tilted cage for 8 h, soiled cage bedding for 8 h, and empty bottle for 8 h. Body weight will be measured once weekly to allow for later assessment of confounds. Sucrose preference test (SPT), open field test (OFT), novel objective recognition test (NORT), Elevated-plus maze (EPM), marble burying test (MBT) and forced swimming test (FST) will be performed from PND 42 to PND 50 (as instructed in Ye et al, 2019). SPT and FST measure depressive-like behaviors, OFT, EPM, and MBT measure anxious behaviors, and NORT measures working memory; each test is considered a stressor for the chronic mild stress paradigm. For validity, scores for these tests may be recorded and compared to those in the study performed by Ye et al. (2019), but for our purposes these are not considered dependent variables.

Anti-inflammatory Analysis

On PND 51, all rats will be anesthetized then decapitated and the blood will be collected in microcentrifuge tubes. After brain removal, the hippocampus and prefrontal cortex will be removed and placed in microcentrifuge tubes. The concentration of IL-10 in the PFC, hippocampus and plasma will be determined using a sandwich ELISA kit from R&D Systems, USA and a microplate reader (as performed in Reus et al, 2017).

Statistical Analysis

Repeated measures of one-way analysis of variance (ANOVA) and Fisher's least significant differences (LSD) post hoc analysis tests will be performed to analyze the significance of any treatment effect among the groups by SPSS, with alpha level set at p=0.05 (Ye et al, 2019).

Expected Results and Interpretation

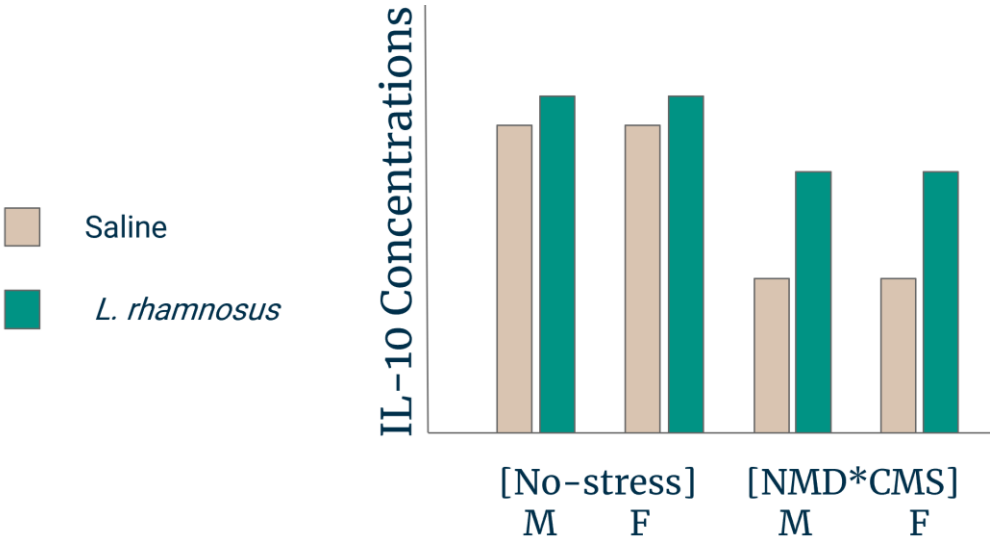

Figure 1: Expected IL-10 concentrations in Sprague-Dawley rats after chronic stress and probiotic treatment. [M=male,F=Female, NMD*CMS=neonatal maternal deprivation and chronic mild stress]

I expect that the expression of IL-10 will be reduced in rats that were exposed to NMD*CMS compared to the rats in the no-stress condition. Additionally, I predict a sex

difference will emerge in anti-inflammatory cytokine levels due to the NMD*CMS condition. That study was conducted with female rats, so male rats may have differing cytokine levels after experiencing the same stressors. This difference is expected to manifest in higher levels of IL-10 in males compared to females.

Rats having received *L. rhamnosus* are expected to have higher levels of IL-10. A sex difference may be seen in IL-10 levels between the NMD*CMS, probiotic and NMD*CMS, saline conditions. Such a result would indicate the efficacy of probiotics as a treatment for inflammation in response to stress.

Limitations of this project would include its generalizability to humans, as the battery of stressors and tests used in the NMD*CMS condition would indicate early life adversity of a high degree. The chronic mild stress does not directly translate to the human experience; however, its validation as a model of ELS justifies its use in the current study. Future directions would include testing multiple probiotics to determine which treatment most effectively moderates the effect of chronic stress in adolescents on inflammation. Probiotic strains could be chosen based on potential human use by testing only those approved by the FDA for human consumption. These could be administered in a sample of human adolescent who have suffered ELS and have been diagnosed with clinical depression. After an effective probiotic is determined, the microbiota effected by that particular strain could be assessed in cases of adolescent depression. To modify the current study, the animal population could be reduced if no significant sex difference is found.

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