NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

A. Haj-Mirzaian et al. / Neuroscience xxx (2017) xxx–xxx

RESEARCH PAPER

Anxiety- and depressive-like behaviors are associated with altered hippocampal energy and inflammatory status in a mouse model of Crohn's disease

Arya Haj-Mirzaian,[a,b†] Shayan Amiri,[a,c†] Hossein Amini-Khoei,[d,e†] Mir-Jamal Hosseini,[f,g] Arvin Haj-Mirzaian,[a,h] Majid Momeny,[a] Maryam Rahimi-Balaei[i] and Ahmad Reza Dehpour[a,h*]

[a] *Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[b] *Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, USA*

[c] *Regenerative Medicine Program, Department of Biochemistry and Medical Genetics, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, MB, Canada*

[d] *Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[e] *Department of Physiology and Pharmacology, School of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[f] *Zanjan Applied Pharmacology Research Center, Zanjan University of Medical sciences, Zanjan, Iran*

[g] *Department of Pharmacology and Toxicology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran*

[h] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[i] *Department of Human Anatomy and Cell Sciences, College of Medicine, Faculty of Health Sciences, University of Manitoba, Winnipeg, Canada*

Abstract—Depression and anxiety are common comorbid disorders observed in patients with inflammatory bowel disease (IBD). Increasing line of evidence indicates that immune-inflammatory responses are involved in co-occurrence of mood disorders and IBD. However, the mechanisms through which immune-inflammatory pathways modulate this comorbidity are not yet understood. This study investigated the role of innate immunity in the development of behavioral abnormalities associated with an animal model of Crohn's disease (CD). To do this, we induced colitis in male adult mice by intrarectal (i.r.) injection of DNBS (Dinitrobenzene sulfonic acid). After 3 days, we performed behavioral tests for anxiety- and depressive-like behaviors as well as tissue collection. Our results showed that DNBS-induced colonic inflammatory responses were accompanied by infiltration of inflammatory cells, and increased expression of genes involved in toll-like receptor signaling pathway in intestinal tissue. Furthermore, the DNBS-treated mice showed depressive- and anxiety-like behaviors which were associated with increased expression of the inflammatory genes and abnormal mitochondrial function in the hippocampus. These results suggest that peripheral inflammation is able to increase the transcriptional level of the genes in toll-like receptor pathway, induces abnormal mitochondrial function in the hippocampus, and these negative effects may be involved in the co-occurrence of anxiety and depression in early stages of CD.

Key words: depression, anxiety, Crohn's disease, oxidative stress, hippocampus, toll-like receptor pathway.

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, is a chronic disease associated with psychiatric comorbidities such as depression and anxiety (Chauhan et al., 2016; Mikocka-Walus et al., 2016). Psychiatric comorbidities in IBD patients negatively affect the quality of their lives and the severity of disease in sufferings (van den Brink et al., 2016; Yanartas et al., 2016). Evidence has shown that the incidence of mood disorders is higher in patients with IBD in comparison with other chronic diseases (Taft and Keefer, 2016). Recent preclinical investigations have revealed that induction of experimental colitis in rodents is able to provoke psychopathologies such as depressive- and anxiety-like behaviors in animals (Bercik et al., 2010). In this regard, it has been shown that dextran sul-

*Correspondence to, A.R. Dehpour: Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box: 13145-784, Tehran, Iran. Fax: +98 2166402569.
E-mail address: dehpour@yahoo.com (A. R. Dehpour).
† Please note that authors contributed equally in this work.

https://doi.org/10.1016/j.neuroscience.2017.10.023
0306-4522/

Please cite this article in press as: Haj-Mirzaian A et al. Anxiety- and depressive-like behaviors are associated with altered hippocampal energy and inflammatory status in a mouse model of Crohn's disease. Neuroscience (2017), https://doi.org/10.1016/j.neuroscience.2017.10.023

fate sodium (DSS)-induced colitis in rodents is accompanied by behavioral comorbidities (Reichmann et al., 2015; Nyuyki-Dufe et al., 2016). Also, interleukin-10 knockout (IL-10−/−) mouse (an animal model for studying IBD) shows anxiety-like behaviors and memory impairments (Kühn et al., 1993; Ohland et al., 2013). Considering direct association between IBD and psychological disorders, only few studies have investigated the underlying mechanisms involved in the comorbidity of IBD and psychiatric disorders.

Emerging line of research suggests that oxidative and nitrosative stress (O&NS) and immune-inflammatory responses play important roles in the pathophysiology of depression (Maes, 2008; Maes et al., 2009, 2011b; Patki et al., 2013). Focusing on IBD, evidence has recently suggested that oxidative challenge and pro-inflammatory cytokines contribute to the pathophysiology of IBD (Ghia et al., 2009; Maes et al., 2011b; Triantafillidis et al., 2013). Evidence also suggests that peripheral inflammation in patients with chronic diseases is able to initiate the development of mood disorders by triggering inflammatory signaling in the brain (Taché and Bernstein, 2009; Bonaz and Bernstein, 2013). Several studies have suggested that gut damage is accompanied by the activation of peripheral inflammatory responses, which may be related to the development of mood disorders in IBD patients (Fleshner, 2013; McCusker and Kelley, 2013; Singhal et al., 2014). Toll-like receptors (TLRs) are the main components of innate immunity, which recognize the pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS) and damage-associated molecular patterns (DAMPs). Activation of TLRs leads to production of pro-inflammatory cytokines such as IL-1β and IL-6, and nitric oxide (NO) in the brain (Barksby et al., 2007; Klune et al., 2008; Diacovich and Gorvel, 2010; Fleshner, 2013). The elevated levels of pro-inflammatory cytokines and nitrosative stress are capable of inhibiting the mitochondrial respiratory chain components and result in cellular energy deficiency (Mancuso et al., 2007; Maes et al., 2011a; Morris and Maes, 2014). Considering the crucial role of mitochondria in massive production of reactive oxygen species (ROS) under pathological conditions, a number of studies suggest that abnormal mitochondrial function and inflammatory responses may contribute to the pathogenesis of depression (Rezin et al., 2009; Bakunina et al., 2015; Crupi and Cuzzocrea, 2016).

In this study, we tested whether (1) experimental model of Crohn's disease is associated with the development of behaviors related to anxiety and depression, and (2) peripheral inflammation following induction of colitis in mice activates the genes relevant to innate immunity and oxidative stress in the hippocampus.

EXPERIMENTAL PROCEDURES

Animals

We used male NMRI mice purchased from the Pasteur Institute, Tehran, Iran. All animals, weighing 25–30 g, were housed in the groups (4 mice in each cage), and were kept at the temperature of 21–23 °C under a 12-h consistent light/dark cycle and were given access to food and water ad lib. All tests were performed between 10:00 and 14:00 h. All procedures were done in line with the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 80-23, revised 1978) and the institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences). All experimental groups have been demonstrated and designated in Table 1. Also, full efforts were made to minimize the use of animals and to optimize their comfort.

Induction and characterization of colitis

Induction of colitis. DNBS (Dinitrobenzene sulfonic acid) (Sigma) was used to induce colitis. For this purpose, 6 mg of DNBS dissolved in 100 μL of 50% ethanol and slowly inject intra-rectally for each mouse. Animals were deprived of food 24 h prior to colitis induction, and were anesthetized using Isoflurane inhalation (Sigma). DNBS was injected intra-rectally (i.r.) using a flexible catheter 5 cm in length. After that the mice were held upside down in a 45° position for 2 min in order to avoid leakage of the DNBS solution, and were returned to their home cages. Animals were assessed for the behavioral or molecular assessments 3 d after colitis induction when the peak of acute inflammation occurs (Hollenbach et al., 2005). Control groups received 100μL normal saline (i.r.) and were evaluated 3 d after injection.

Macroscopic assessment. Animals were euthanized under deep anesthesia using isoflurane inhalation (3 d after DNBS or saline injection), colon was dissected out and cut open longitudinally and gently cleaned using PBS. The assessment of inflammation was scored based on ulceration, inflammation, and extent of disease. The scoring system subordinates from following scale from 0 to 9: 0 = normal aspect of the mucosa, 1 = localized hyperemia without ulcerations, 2 = ulceration, 3 = ulceration with thickening of bowel wall at one site, 4 = two or more sites of ulceration and thickening of the bowel wall, 5 = major sites of damage extending < 2 cm along the length of the colon, and 6–9 = damage extending > 2 cm (with the score increasing by 1 for each centimeter of damaged tissue) (El-Salhy et al., 2014).

Histopathological evaluation. In order to microscopic assessments, the colon was cut into pieces and fixed in 10% formalin. Formalin-fixed colon segments were paraffin-embedded and cut into 5-μm divisions. Nine sections obtained from each colon and were deparaffinized using xylene and stained with hematoxylin and eosin (H&E) (Hasanvand et al., 2016). Histological analysis was done under light microscopy by a pathologist in consistent with the previously described method (Obermeier et al., 1999). Each score characterized the mean of nine sections of each colon.

Please cite this article in press as: Haj-Mirzaian A et al. Anxiety- and depressive-like behaviors are associated with altered hippocampal energy and inflammatory status in a mouse model of Crohn's disease. Neuroscience (2017), https://doi.org/10.1016/j.neuroscience.2017.10.023