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Research report

Metabolomic analysis reveals metabolic disturbances in the prefrontal cortex of the lipopolysaccharide-induced mouse model of depression

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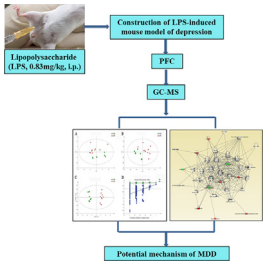
HIGHLIGHTS

- Successfully constructed the LPS-induced mouse model of depression.
- GC–MS-based metabolic profiling was conducted.
- A total of 20 differential metabolites were identified.
- Differentially expressed metabolites are mainly involved in amino acid, lipid, energy, and oxidative stress metabolism.

GRAPHICAL ABSTRACT



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ABSTRACT

Major depressive disorder (MDD) is a debilitating illness. However, the underlying molecular mechanisms of depression remain largely unknown. Increasing evidence supports that inflammatory cytokine disturbances may be associated with the pathophysiology of depression in humans. Systemic administration of lipopolysaccharide (LPS) has been used to study inflammation-associated neurobehavioral changes in rodents, but no metabonomic study has been conducted to assess differential metabolites in the prefrontal cortex (PFC) of a LPS-induced mouse model of depression. Here, we employed a gas chromatography–mass spectrometry-based metabonomic approach in the LPS-induced mouse model of depression to investigate any significant metabolic changes in the PFC. Multivariate statistical analysis, including principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA), and pair-wise orthogonal projections to latent structures discriminant analysis (OPLS-DA), was implemented

Abbreviations: BW, body weight; CNS, central nervous system; CUMS, chronic unpredictable mild stress; DLPFC, dorsolateral prefrontal cortex; FST, forced swimming test; GABA, γ-aminobutyric acid; GC–MS, gas chromatography–mass spectrometry; HMDB, human metabolome tatabase; IPA, ingenuity pathways analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; LC–MS, liquid chromatography–mass spectrometry; LPS, lipopolysaccharide; MDD, major depressive disorder; NMR, nuclear magnetic resonance; OPLS-DA, orthogonal partial least-squares discriminant analysis; PBMCs, peripheral blood mononuclear cells; PCA, principal component analysis; PFC, prefrontal cortex; PLS-DA, partial least squares-discriminate analysis; RT, retention time; SEM, standard errors of the mean; SPT, sucrose preference test; TIC, total ion current; TST, tail suspension test; VIP, variable influence on projection.

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Gas chromatography–mass spectrometry

to identify differential PFC metabolites between LPS-induced depressed mice and healthy controls. A total of 20 differential metabolites were identified. Compared with control mice, LPS-treated mice were characterized by six lower level metabolites and 14 higher level metabolites. These molecular changes were closely related to perturbations in neurotransmitter metabolism, energy metabolism, oxidative stress, and lipid metabolism, which might be evolved in the pathogenesis of MDD. These findings provide insight into the pathophysiological mechanisms underlying MDD and could be of valuable assistance in the clinical diagnosis of MDD.

1. Introduction

Major depressive disorder (MDD) is a debilitating mood disorder with a lifetime prevalence of 4.4% to 20% in the general population. It is an increasing public health issue that adversely affects social interaction and general health globally. Depressive disorders are common forms of psychiatric illness, which are predicted to become the second leading cause of disability by the year 2020 [1,2]. There have been various etiopathological hypotheses regarding depression such as monoamine deficiency, neurogenesis, hypothalamic pituitary adrenal axis dysfunction [3], and imbalance of glutaminergic neurotransmitter system hypotheses [4]. However, depression is a heterogeneous disorder with a highly variable course, and none of the established hypotheses can independently account for the complex pathogenesis of MDD.

Increasing evidence supports the view that inflammatory cytokine disturbances may be associated with the pathophysiology of depression in humans [5,6]. A model of depression has been established in laboratory animals with low doses of lipopolysaccharide (LPS), which is an efficient and vigorous inducer of inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6). LPS acutely activate the peripheral or central innate immune system to trigger depressive-like behavioral alterations [6,7]. The LPS-induced mouse model of depression, which has been generally adopted for elucidating the relationship between of the immune system and depressive symptoms, has repeatedly proved to be an effective and predictive animal model of depression [8]. Following exposure to LPS [9], mouse depressive behaviors are reflected in increased immobility in the forced swimming test (FST) and tail suspension test (TST), and in a reduced preference for sweet solutions, as assessed by the sucrose preference test (SPT). Congruently, antidepressants have been found to improve cytokine-evoked depressive-like behaviors in animals [10]. Therefore, it appears that elevated inflammatory cytokine levels are associated with the development of depression. However, the mechanisms underlying this phenomenon remain largely unknown.

Recent studies have pointed towards the prefrontal cortex (PFC) playing a significant role in regulating emotion, memory, cognition, and learned responses [11,12]. A large number of functional imaging, lesion, and brain stimulation studies have implicated the PFC in the pathophysiology of neuropsychiatric disorders[13,14], including inflammation related disorders such as depression [15]. For instance, MDD patients show gray matter density abnormalities in the right dorsolateral prefrontal cortex (DLPFC) [16]. Also, depressed patients have significantly decreased cerebral blood flow and glucose metabolism in the PFC, accompanied by a corresponding reduction in PFC grey matter volume [17]. Veeraiah et al. reported that glutamatergic and γ-aminobutyric acidergic (GABAergic) activities are dysfunctional in the PFC of mice in a social defeat model of depression [18]. An imbalance of left-right DLPFC in MDD has been linked to negative emotional judgment through functional magnetic resonance imaging [19]. Furthermore, high-frequency repetitive transcranial magnetic stimulation of the left DLPFC has been shown to be effective in treatment-resistant depression [20]. Thus, the PFC may also be implicated in the pathophysiology of depression.

Our lab has been focusing on MDD for many years [9,21–23]. Our research group has previously performed proteomic research on a chronic unpredictable mild stress (CUMS) rat model of depression and a LPS-induced mouse model of depression that revealed a perturbation of energy in the PFC [9,24]. However, proteomic data may only partially indicate pathophysiological effects, because many pathway feedback mechanisms are not reflected in protein concentration changes [25]. Recently, metabolomics, which is a quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification, has emerged. This is an efficient approach for probing significant biochemical alterations, exploring potential pathophysiological mechanisms, and evaluating the therapeutic effects of drugs [25]. Currently, metabolomics have been widely used not only in the peripheral area such as plasma [26] and urine [27], but also in the central nervous system (CNS) [28]. Metabolomic profiling methods, such as gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS), and nuclear magnetic resonance (NMR), have captured considerable pathophysiological changes in MDD in both animal models and patients. Compared with other methods, GC–MS has been widely used because of its high sensitivity, peak resolution, and reproducibility. Based on previous studies by our group, we believe there is mounting evidence of metabolic irregularities in peripheral blood mononuclear cells (PBMCs) [29] and brain tissue [30,31] in the CUMS depression model, and in the urine [21,27] and plasma [32] of patients with MDD. However, no reports regarding metabolic abnormalities of in PFC tissue in an inflammation-related mouse model of depression have been published thus far.

Therefore, in the present study, we constructed a well-validated LPS-induced mouse model of depression [7,9,33]. We employed a GC–MS metabolomic approach coupled with principal component analysis (PCA), partial least squares-discriminate analysis (PLS-DA), and orthogonal partial least-squares discriminant analysis (OPLS-DA) to comparatively analyze significant metabolic changes in the PFC's of LPS-induced depressed and healthy mice. The primary objective of this study was to elucidate the effects of LPS on PFC metabolic profiles in order to better understand the underlying mechanisms of depression.

2. Materials and methods

2.1. Animals and ethics statement

Adult 12-week-old male CD-1 (ICR) mice (specific-pathogen-free grade) weighing 35–40 g at the beginning of the experiment were obtained from the animal facility at Chongqing Medical University (Chongqing, China). This study was approved by the Ethics Committee of Chongqing Medical University. The handling, experimental procedures, and care of all animals were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised