Behavioural Brain Research 317 (2017) 263–271

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Lentinan produces a robust antidepressant-like effect via enhancing the prefrontal Dectin-1/AMPA receptor signaling pathway

Hongkun Bao[a,b,1], Lijuan Sun[a,b,1], Ying Zhu[a,c,1], Pengzhan Ran[a,b], Weihong Hu[c], Keming Zhu[a,b], Bai Li[a,b], Yangyang Hou[a,b], Jun Nie[a,b], Tangxin Gao[a,b], Liping Shan[d], Kai Du[e], Shangyong Zheng[b], Bingrong Zheng[b], Chunjie Xiao[a,b,*], Jing Du (MD PhD) (Professor)[a,b,**]

[a] State Key Laboratory for Conservation and Utilization of Bio-resources in Yunnan, Yunnan University, Kunming, Yunnan, PR China
[b] School of Medicine, Yunnan University,Kunming, Yunnan, PR China
[c] School of Life Sciences, Yunnan University, Kunming, Yunnan, PR China
[d] Beijing Gragen Biotechnology Co. Ltd., Beijing, PR China
[e] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, PR China

HIGHLIGHTS

- Lentinan treatment led to a robust antidepressant-like effect in the tail suspension test and forced swim test after 60 min of treatment in mice.
- Dectin-1, the receptor of lentinan, mediated the antidepressant-like effects of lentinan.
- The prefrontal Dectin-1/AMPA receptor signaling pathway was essential for the robust antidepressant-like effects evoked by lentinan.

ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
24 September 2016
Accepted 27 September 2016

Keywords:
Lentinan
Dectin-1
Animal behavior
GluR1
Antidepressant
AMPA receptor

ABSTRACT

Lentinan (LNT) is an immune regulator and its potential and mechanism for the treatment of mood disorder is of our interest. Dectin-1 is a β-glucan (including LNT) receptor that regulates immune functions in many immune cell types. Cumulative evidence has suggested that the glutamatergic system seems to play an important role in the treatment of depression. Here, we studied the antidepressant-like effects of LNT and its therapeutical target in regulating the functions of AMPA receptors. We found that 60 min treatment with LNT leads to a significant antidepressant-like effect in the tail suspension test (TST) and the forced swim test (FST) in mice. The antidepressant-like effects of LNT in TST and FST remained after 1 day or 5 days of injections. Additionally, LNT did not show a hyperactive effect in the open field test. Dectin-1 receptor levels were increased after LNT treatment for 5 days and the specific Dectin-1 inhibitor laminarin was able to block the antidepressant-like effects of LNT. After 5 days of treatment, LNT enhanced p-GluR1 (S845) in the prefrontal cortex (PFC); however, the total GluR1, GluR2, and GluR3 expression levels remained unchanged. We also found that the AMPA-specific blocker GYKI 52466 was able to block the antidepressant-like effects of LNT. This study identified LNT as a novel antidepressant with clinical potential and a new antidepressant mechanism for regulating prefrontal Dectin-1/AMPA receptor signaling.

Abbreviations: LNT, lentinan; TST, tail suspension test; FST, forced swim test; OFT, open field test; PFC, prefrontal cortex; MDD, Major depressive disorder; Dectin-1, Dendritic cell-associated C-type lectin-1; p-GluR1(S845), phosphorylation of GluR1 S845; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; Lam, laminarin; NMDA, non-competitive N-methyl-D-aspartate; i.p., intraperitonial; mins, minutes; IMI, imipramine; BBB, blood brain barrier; TLR2, toll-like receptor 2; Sal, saline.

* Corresponding authors at: State Key Laboratory for Conservation and Utilization of Bio-resources in Yunnan, Yunnan University, Kunming, Yunnan, PR China.
** Correspondence to: Yunnan University, School of Medicine, 2 Cuihu North Road, Kunming, Yunnan, PR China.
E-mail address: dujing@ynu.edu.cn (J. Du).
[1] These three authors contributed equally to the paper as first authors.

http://dx.doi.org/10.1016/j.bbr.2016.09.062
0166-4328/

1. Introduction

Major depressive disorder (MDD), a common mental disorder, is the leading cause of disability and a major contributor to disease burden in global population [1]. Lentinan (LNT) possesses a wide range of beneficial effects with therapeutic potential, including anti-inflammatory, anti-cancer, and immune-regulatory activities [2–5]. Dendritic cell-associated C-type lectin-1 (Dectin-1) is an immune regulator and receptor for lentinan, a purified β-1, 3-glucan with β-1, 6-branches that is derived from the edible mushroom *Lentinus edodes (Berk) Sing* [6,7]. It is a β-glucan receptor that is present in leukocytes and has the highest levels of cell surface expression in neutrophils, macrophages, and dendritic cells [8]. Particularly, Dectin-1 was found in microglial cells in the central nervous system [9], suggesting its role in regulating neuroimmune system. Dectin-1 was discovered as the first non-Toll-like receptor. Activation of Dectin-1 triggers the expression of cytokines and an increase of phagocytosis [10,11]. Cumulative evidence has suggested that immune cells and their signaling play a major role in the pathophysiology of MDD [12,13]. However, whether LNT or/and its receptor Dectin-1 are related to this antidepressant-like effects remains unclear.

Glutamate mediates approximately 80% of the excitatory synaptic transmission in the central nervous system (CNS) [14,15]. Recently, the glutamatergic system is identified as the central mediator for the pathophysiology and treatment of depression [15–18]. The most famous rapid antidepressant, ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, was found to rely on increasing α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) signaling to exert its antidepressant efficacy [19,20]. It has been shown that the AMPA potentiator demonstrated an antidepressant-like effect in the animal models of depression [21]. It has been shown in the clinical studies that the expression of AMPA GluR1 receptor was decreased in the brain of patients with depression [22,23]. Moreover, phosphorylation of the AMPA receptor subunit GluR1 is the key regulator for the trafficking of the GluR1 into the functional synapses [24,25]. Following traditional antidepressant treatment with flouxetine or tianeptine, phosphorylation of GluR1 at serine 845 (S845) was increased in mice [26,27]. Previous studies suggested that phosphorylation of GluR1 S845 [p-GluR1(S845)] was associated with the wide opening of the AMPA receptor channels and increased localization of AMPA receptors at the synapses [25,28–31], which is important for the treatment of depression. However, whether LNT regulates AMPA synaptic plasticity in the prefrontal cortex (PFC) is still unknown.

Based on these premises, we designed a series of behavioral and biochemical experiments to investigate the antidepressant-like effects of LNT in animal models of depression. We studied the effects of various concentrations of LNT on animal models of depression, including the tail suspension test (TST), forced swim test (FST) and open field test (OFT). The involvement of Dectin-1 and toll-like receptor 2 (TLR2) in the antidepressant-like effects of LTN was tested. The phosphorylation of AMPA GluR1 S845 and AMPA GluR1, GluR2, and GluR3 were determined in the PFC after 5 days of treatment. In addition, the role of the enhanced AMPA function in the antidepressant-like effects was addressed by treatment with the AMPA-specific antagonist GYKI 52466 followed by the TST.

2. Materials and methods

2.1. Animals

Male CD-1 mice (24–26 g; Vital River, Beijing, China) were group housed (N = 4/cage) in an animal room with a constant temperature ($23 \pm 1\,^{\circ}C$) and maintained on a 12 h/12 h light/dark cycle with constant humidity ($50 \pm 10\%$) and free access to water and food. After a one-week acclimatization period, the mice were treated with drugs or vehicle in a volume of 10 μl/g of body weight by intraperitonial (i.p.) injection. The experiments were carried out between 10:00 a.m. and 2:00 p.m. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (ISBN: 0-309-05377-3) [32] and were approved by the Institutional Animal Care and Use Committee at Yunnan University, School of Medicine (IACUC: MS201402).

2.2. Experimental design

To determine the antidepressant-like effects of LNT (FuSheng Inc. Ltd., Shanghai, China), mice were randomly assigned to five treatment groups, saline (0.9% sterile sodium chloride solution), low dose of LNT (5 mg/kg, dissolved in saline), medium dose of LNT (12.5 mg/kg, in saline), high dose of LNT (20 mg/kg, in saline), and imipramine (15 mg/kg, in saline, Sigma, St. Louis, MO, USA). All animal behavior tests were performed at 60 min after the drug or vehicle administration. TST or FST was performed at 60 min, 1 day or 5-consecutive-day under the similar settings, and OFT was performed on the fourth day after the injection.

To determine whether the antidepressant-like effects of LNT could be blocked by laminarin (Lam, a specific Dectin-1 blocking reagent, Sigma, St. Louis, MO, USA), CD-1 mice were i.p. injected with laminarin (10 mg/kg) 2 h prior to the behavioral testing, and this was followed one hour after the LNT injection. Sixty minutes after the LNT injection, the mice were subjected to the TST [33]. The AMPA-specific blocker GYKI 52466 (a selective non-competitive AMPA receptor antagonist, TOCRIS Bioscience, R&D, Minneapolis, USA) was used to determine the AMPA effect. Mice were treated with LNT on the first day and the second day (60 min before the TST). GYKI 52466 (15 mg/kg in 16% DMSO/84% saline) or vehicle was administered 30 min before the TST [34]. For each drug treatment, the control mice received the respective vehicle alone.

2.3. Tail suspension test (FST)

The tail suspension test was performed according to the previous procedure [35]. Mice were suspended by the tail 50 cm above the floor by adhesive tape placed approximately 1 mm from the tip of the tail. During the test, no mice climbed their tails. Each mouse was individually videotaped during a 6 min test session. The immobility time was quantified by a naive observer for the last 4 min of the 6 min session.

2.4. Open field test (OFT)

A chamber ($60 \times 60 \times 30$ cm) with a black floor that was divided into 16 squares of equal area (15×15 cm) by white lines was used to study LNT-induced locomotor hyperactivity. After 4 days of i.p. injection with various concentrations of drugs, mice were placed in the center of the chamber and their behavior was recorded for 60 min. The total distance traveled and the amount of distance traveled in the center area (the 4-square area in the middle of the chamber) were analyzed by the ANY-maze software (Stoelting, Wood Dale, IL, USA).

2.5. Forced swim test (FST)

The forced swim test was carried out according to previously report [36]. Mice were placed individually into a plastic cylinder (40 cm high and 20 cm in diameter) with water ($23 \pm 1\,^{\circ}C$), which was 20 cm deep. Mice were videotaped during a 6 min test session, and the mice spent immobility time was quantified by a naive