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Honokiol abrogates lipopolysaccharide-induced depressive like behavior by impeding neuroinflammation and oxido-nitrosative stress in mice

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ABSTRACT

Depression is an inflammatory, commonly occurring and lethal psychiatric disorder having high lifetime prevalence. Preclinical and clinical studies suggest that activation of immuno-inflammatory and oxido-nitrosative stress pathways play major role in the pathophysiology of depression. Honokiol (HNK) is a biphenolic neolignan possessing multiple biological activities including antioxidant, anti-inflammatory, anxiolytic, antidepressant and neuroprotective. The present study investigated the effect of HNK (2.5 and 5 mg/kg, i.p.) pretreatment (30 min prior to LPS) on lipopolysaccharide (LPS) (0.83 mg/kg, i.p.) induced depressive like behavior, neuroinflammation, and oxido-nitrosative stress in mice. HNK pretreatment at both the doses significantly attenuated LPS induced depressive-like behavior by reducing the immobility time in forced swim and tail suspension test, and by improving the anhedonic behavior observed in sucrose preference test. HNK pretreatment ameliorated LPS induced neuroinflammation by reducing IL-1β, IL-6 and TNF-α level in hippocampus (HC) and prefrontal cortex (PFC). HNK pretreatment prevented LPS evoked oxidative/nitrosative stress via improving reduced glutathione level along with reduction in the lipid peroxidation and nitrite level in HC and PFC. Pretreatment with HNK also prevented the increase in plasma corticosterone (CORT) and decrease in hippocampal BDNF level in LPS challenged mice. In conclusion, current investigation suggested that HNK pretreatment provided protection against LPS-induced depressive like behavior which may be mediated by repression of pro-inflammatory cytokines as well as oxido-nitrosative stress in HC and PFC. Our results strongly speculated that HNK could be a therapeutic approach for the treatment of depression and other pathophysiological conditions which are closely associated with neuroinflammation and oxido-nitrosative stress.

1. Introduction

Depression is a debilitating, commonly occurring and persistent recurring psychiatric disorder resulting in vast personal suffering that lead to socio-economic burden. Globally, it is the third most important cause of disability and projected to be the largest contributor to the burden of disease by the year 2030 (WHO, 2008). It is affecting 350 million people worldwide with lifetime prevalence in the range of 1.5–19.0% (Bromet et al., 2011). About half of the depressed patients are unaware of their disease, or misdiagnosed or not treated effectively which further increases the burden of this disease. At present, most of the clinically used antidepressants are of synthetic origin and generally takes 6–8 weeks to produce noticeable therapeutic response (Uher et al., 2011). Unfortunately, antidepressants are also inundated by side effects, slow onset of actions, and drug–drug interactions (Nestler et al., 2002). Heterogeneity of depressive symptoms among the patients and inconsistent therapeutic responses of the present antidepressants raise a need to identify novel antidepressant which would have higher efficacy and fewer side effects.

Several experimental studies advocate that activation of immune-inflammatory and oxido-nitrosative stress pathways leads to develop depression (Maes, 2008). High levels of pro-inflammatory cytokines

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such as TNF-α, IL-6, and IL-1β in serum or plasma have been found in depressed patients (Hannestad et al., 2011). Inflammatory challenge through peripherally or centrally by lipopolysacchride (LPS) exhibits both sickness and depressive-like behavior in animal model. LPS elicits a systemic inflammation through increase in the expression of pro-inflammatory mediators, such as TNF-α, IFN-γ, IL-6, and IL-1β (Godbout et al., 2005; Huang et al., 2008). These pro-inflammatory cytokines generates oxidative stress condition which may further lead to rapid alteration in the antioxidant system (Sugino et al., 1987). Neuroinflammation along with oxido-nitrosative stress perpetuates a vicious cycle that ultimately leads to behavioral alterations.

Honokiol (3,5-di-2-propenyl-1,1-biphenyl-2,4-diol) is a biphenolic bioactive component present in the stem bark of *Magnolia officinalis*. Recent in vivo and in vitro studies have demonstrated multiple biological properties of honokiol (HNK) such as antioxidant, anti-inflammatory, anti-arrhythmic, anti-tumor, anti-angiogenic, cardioprotective, antimicrobial, and antifibrotic (Arora et al., 2011; Chao et al., 2010; Chiang et al., 2011; Dikalov et al., 2008; Fried and Arbiser, 2009; Hu et al., 2005; Li et al., 2011b; Munroe et al., 2007; Park et al., 2004; Tsai et al., 1999; Wang et al., 2011, 2013; Zhao and Liu, 2011). Previous studies demonstrated the anti-inflammatory roles of HNK in dendritic cells, macrophages and several other cellular systems (Chiang et al., 2011; Li et al., 2011a, 2011b; Lin et al., 2007; Wang et al., 2013). HNK potentially exerts its anti-inflammatory effect via acting on different pathways like phosphoinocytide-3 kinase/AKT (protein kinase/AKT), p38, extracellular signal-regulated kinases 1/2 (ERK1/2) as well as c-Jun N-terminal kinases 1/2 (JNK1/2) (Chao et al., 2010; Kim and Cho, 2008; Li et al., 2011a). Recently, a study focusing on the use of HNK on gliosarcoma reveals that it can easily cross the blood-brain barrier and blood-cerebrospinal fluid barrier, which makes it a drug of choice for CNS disorders (Wang et al., 2011). In addition, HNK does not alter the expression of toll-like receptor-4 at surface and binding of LPS to its receptors (Li et al., 2011a). In addition, various previous studies claimed that HNK possesses anxiolytic, antidepressant and neuroprotective properties (Chen et al., 2007; Kuribara et al., 1999; Qiang et al., 2009; Xu et al., 2008).

Thus, considering the anti-inflammatory and antioxidant properties of HNK as well as its easy penetrability into the brain, we hypothesized that HNK pretreatment would result in protective effect against LPS-induced depressive-like behavior and neurochemical alterations in mice. Furthermore, we assessed whether the possible antidepressant-like effects of this compound are associated with alterations in IL-1β, IL-6, TNF-α and BDNF levels, along with changes in oxido-nitrosative stress parameters in PFC and HC areas of the mice brain following an immune challenge with LPS.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide from *Escherichia coli* (L-3129, serotype 0127: B8), honokiol, Griess reagent, Thiobarbituric acid, 5, 5′-dithiobis (2-nitrobenzoic acid), and L-Reduced glutathione were purchased from Sigma-Aldrich, St. Louis, MO, USA. Interleukin1-β, Interleukin-6, and Tumor Necrosis Factor (TNF)-α immunoassay kits were purchased from Invitrogen Co., Carlsbad, CA, USA. All other chemicals were of analytical grade and purchased from Sigma-Aldrich Chemicals (Saint Louis, MO, USA) unless mentioned otherwise.

2.2. Animals

Adult male Swiss albino mice (22–30 g) were used for the study. Standard laboratory animal feed (Pranav Agro Industries Ltd., Pune, India) and water were provided ad libitum. All animal experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, between 08:00 h and 14:00 h. The animal experimentation protocols were approved by the Institutional Animal Ethics Committee (IAEC), Gauhati Medical College and Hospital (CPCSEA Registration no.351, 3/1/2001). Animals were acclimatized to the laboratory conditions one week prior to the start of experiments.

2.3. Preparation of doses

Fresh solutions of LPS and honokiol (HNK) were prepared daily from 1 mg/ml stock solutions. LPS was dissolved in sterile, endotoxin-free normal saline and injected intraperitoneally at the dose of 0.83 mg/kg BW. The selected dose of LPS was chosen based on its ability to induce a transient sickness behavior response followed by the development of a distinct depressive-like behavioral phenotype in the forced swim and tail suspension tests (O'Connor et al., 2009). Different doses of HNK (2.5, 5 mg/kg BW) were selected based on the previous experimental study and preliminary studies performed in our laboratory. HNK was dissolved in DMSO:PBS (1:1) and administered intraperitoneally (i.p.) (Kaushik et al., 2012).

2.4. Experimental design

In the present study, animals were divided into six experimental groups ($n=6$–10 mice/group) for behavioral and biochemical assessment. Subsequent treatments were as follows: (1) Group I was treated with vehicle [DMSO:PBS (1:1)] of HNK, 30 min prior to saline administration. This group served as control group. (2) Group II was treated with vehicle [DMSO:PBS (1:1)] of HNK, 30 min prior to LPS (0.83 mg/kg, i.p.) challenge. This group served as LPS control group. (3) Group III was treated with HNK (2.5 mg/kg, i.p.), 30 min prior to LPS (0.83 mg/kg, i.p.) challenge. (4) Group IV was treated with HNK (5 mg/kg, i.p.), 30 min prior to LPS (0.83 mg/kg, i.p.) challenge. (5) Group V was treated with HNK (2.5 mg/kg, i.p.), 30 min prior to saline injection. (6) Group VI was treated with HNK (5 mg/kg, i.p.), 30 min prior to saline injection (Fig. 1).

Various behavioral parameters such as open field test, forced swim test, sucrose preference test were assessed after 24 h of LPS administration. Tail suspension test was conducted after 28 h of LPS administration. Biochemical and behavioral parameters were performed by taking different animal groups. Plasma corticosterone level was estimated after 4 h of saline or LPS challenge.

2.5. Behavioral assessment

2.5.1. Open-field test (OFT)

The open field test was performed before the formal experiments. This test is used for assessment of behavioral changes in rodents exposed to novel environments and is used to confirm that the observed antidepressant effect is not due to stimulation of general motor activity (Santosh et al., 2011). Mice were individually placed into a clean, novel cage similar to the home cage, but devoid of bedding or litter. The cage was divided into nine virtual quadrants, and locomotor activity was measured by counting the number of line crossings and rearing during a 5 min period.

2.5.2. Forced swimming test (FST)

The forced swimming test was performed to assess the despair behavior of the rodents (Porsolt et al., 1977). The forced swim test for mice consisted of cylinder (diameter 15 cm, height 25 cm) containing 15 cm of water maintained at 25 ± 1 °C. Mice were placed in an