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Honokiol abrogates chronic restraint stress-induced cognitive impairment and depressive-like behaviour by blocking endoplasmic reticulum stress in the hippocampus of mice

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ABSTRACT

The primary objective of our study is to investigate the neuroprotective efficacy of honokiol and imipramine against restraint stress (RS)-induced cognitive impairment and depressive-like behaviour in mice. We examined whether the neuroprotective activity of honokiol and imipramine mediates through the inhibition of endoplasmic reticulum stress. Adult Swiss albino mice were restrained for 6 h/day for 28 days. Honokiol (3 and 10 mg/kg) and Imipramine (10 and 30 mg/kg) were administered for last 7 days to the different groups. Cognitive function was assessed by Morris water maze and novel object recognition test. Forced swimming test and tail suspension test were performed to evaluate the restraint stress-induced depressive-like behaviour. Proinflammatory cytokines, brain-derived neurotrophic factor, and ER stress markers i.e. 78-kDa glucose-regulated protein (GRP78) and C/EBP homologous protein (CHOP) were quantified in the hippocampus. We observed cognitive impairment and depressive-like behaviour in RS-exposed animals. Honokiol (10 mg/kg) treated group depicted marked reduction in cognitive impairment and depressive-like behaviour. However, imipramine (10 and 30 mg/kg) prevented the depressive-like behaviour but failed to prevent RS-induced cognitive impairment. Moreover, proinflammatory cytokines, GRP78 and CHOP were elevated in the hippocampus of stressed mice as compared to unstressed mice. Honokiol (10 mg/kg) significantly prevented the RS-induced elevated levels of proinflammatory cytokines and endoplasmic reticulum stress markers. Our results clearly suggest the beneficial potential of honokiol in restraint stress through inhibition of proinflammatory cytokines and endoplasmic reticulum stress. Honokiol could be an intriguing therapeutic approach in endoplasmic reticulum stress related neuro-pathophysiological conditions.

1. Introduction

Stress is considered as one of the crucial risk factors for the development of mental illness such as depression and cognitive dysfunction. Repeated and prolonged stress exposure leads to activation of hypothalamic-pituitary-adrenal axis (HPA) and sympatho-adrenomedullary system (SAM). Thereafter, hyperactivity of HPA axis elevates the level of corticosterone which further affects the cognitive function (Harvey et al., 2006). On the other hand, SAM system increases the catecholamines level to strengthen the fear conditioning during stress (Arnsten, 2009). Studies have proved that chronic restraint stress in the rodents results in neurobehavioural abnormalities such as anxiety, depressive illness as well as cognitive impairment (Ferraz et al., 2011; Kim and Han, 2006; Tian et al., 2013; Vyas and Chattarji, 2004). In addition, restraint stress can also trigger oxidative/nitrosative stress, proinflammatory cytokines release, upregulate Cyclooxygenase-2 (COX-2) expression and activate transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in different brain regions (Fontella et al., 2005; Madrigal et al., 2002; Madrigal et al., 2003; Munhoz et al., 2008; Voorhees et al., 2013). Accumulating evidences suggest that chronic and repeated restraint stress exposure leads to alteration in hippocampal volume, morphology and function (McLaughlin et al., 2007; Zhang et al.,

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2014). Chronic stress-induced neuronal cell loss and apoptotic cell death in the hippocampal region is primarily responsible for the development of depressive illness and cognitive impairment (Lucassen et al., 2006; Zhang et al., 2014).

The endoplasmic reticulum (ER) is a central subcellular organelle where secretory and membrane proteins are synthesised and folded. ER is highly developed in the neuronal cells. Therefore ER is susceptible to various pathophysiological conditions such as hypoxia, stress, hypoglycemia, calcium depletion, oxidative stress, and high-fat diet which can elicit ER stress (Kim et al., 2008; Zhang et al., 2014; Zhao et al., 2013). ER stress can simply be defined as an imbalance between protein folding load and capacity of ER. To overcome ER stress, the unfolded protein response (UPR) is invoked by the activation of several signalling pathways; this UPR promotes an adaptive response to ER stress and re-establishes homeostasis in the ER (Rasheva and Domingos, 2009; Ron and Walter, 2007). Molecular chaperones such as the 78-kDa (GRP78) and the 94-kDa glucose regulated protein (GRP94) are induced during UPR and promote correct protein folding. If the damage is too severe to repair, C/EBP-homologous protein (CHOP) and other factors are activated and induce cell apoptosis (Araki et al., 2003). CHOP-mediated neuronal cell death is implicated in various neurodegenerative diseases (Oyadomari and Mori, 2004; Zhang et al., 2013). Recent studies reported the involvement of ER stress in restraint stress and chronic social defeat stress-induced depressive-like behaviour and cognitive impairment (Zhang et al., 2014; Zhao et al., 2013). This suggests the possibility of an ER stress involvement in the pathogenesis of stress-related behavioural alterations.

Honokiol is a natural bioactive biphenolic lignan (chemically known as 3, 5-di-2-propenyl-1, 1-biphenyl-2, 4-diol) which was first isolated and identified from the stem bark of *Magnolia obovata* Thunb (Fujita et al., 1973). Honokiol can readily cross the blood brain barrier and the blood-cerebrospinal fluid barrier, so assumed to be a potent therapeutic compound with high bioavailability against neurological disorders. Previous experimental reports demonstrated the antioxidative, anti-inflammatory, antiarrhythmic, anti-tumour, anti-angiogenic, cardioprotective, antimicrobial, antifibrotic and antidepressant activities of honokiol (Arora et al., 2012; Chen et al., 2010; Sulakhiya et al., 2014). It has been reported that honokiol ameliorates testicular toxicity via inhibition of ER stress-related apoptosis (Huang et al., 2012)

In the present study, we aimed to investigate the involvement of ER stress-related genes (GRP78 and CHOP) expression upon exposure of chronic restraint stress to mice for 28 days. We hypothesized that honokiol treatment can ameliorate the chronic restraint stress-induced depressive illness and cognitive impairment.

2. Material and methods

2.1. Animals

Healthy adult male Swiss albino mice weighing between 25 ± 3 g were purchased from College of Veterinary Science, Khanapara, Assam. The experimental protocol was approved (approval no. MC/32/2013/36) by the Institutional Animal Ethics Committee (IAEC), Guwahati Medical College & Hospital (CPSCEA Registration No. 351;3/1/2001). The animals were housed in a group (4 in each cage) in a room under controlled environment with temperature (25 ± 1 °C), humidity ($65 \pm 10\%$) and 12 h light–12 h dark cycle. Food and water were provided *ad libitum* and uniform conditions such as temperature and humidity were maintained throughout the experiment. The behavioural testing was performed in dimly lit room. The animals were acclimatized for two weeks prior experiments.

2.2. Drugs and chemicals

Honokiol and imipramine were purchased from Sigma Aldrich were dissolved in 10% dimethyl sulfoxide solution and water respectively. Primers for real-time PCR were purchased from ILS primers India, RNA isolation kit was purchased from Himedia, India. cDNA synthesis kit, and ELISA kits of IL-1β and TNF-α were purchased by Thermoscientific, India. All other chemicals used were of commercial grade.

2.3. Experimental design

The animals were randomly divided into six groups ($n=8$). Subsequent treatments were as followed:

- Group 1 was not provided with any kind of stress but administered vehicle for 7 days before behavioural and biochemical estimation. This group was assigned as normal control group.
- Group 2 animals were restrained (6 h/day for 28 days) but no drug or vehicle treatment was provided. This group was assigned as restraint control.
- Group 3 animals were restrained (6 h/day for 28 days) and treated with imipramine (10 mg/kg, i.p.) for last 7 days of restraint stress.
- Group 4 animals were restrained (6 h/day for 28 days) and treated with imipramine (30 mg/kg, i.p.) for last 7 days of restraint stress.
- Group 5 animals were restrained (6 h/day for 28 days) and treated with Honokiol (3 mg/kg, i.p.) for last 7 days of restraint stress.
- Group 6 animals were restrained (6 h/day for 28 days) and treated with Honokiol (10 mg/kg, i.p.) for last 7 days of restraint stress.

The study plan is graphically described in Fig. 1. The animals were restrained for 6 h (09:00–15:00) daily, up to 28 consecutive days using 50 ml polystyrene tubes. In our preliminary study, we observed 21 days stress exposure caused depressive-like behaviour, spatial memory impairment but recognition memory was unaffected. So, we continued our restraint stress protocol up to 28 days and thereafter we found there was significant impairment in recognition memory along with spatial memory. Therefore, we followed 28 days restraint stress protocol. The 28 days of restraint stress protocol was followed in many previous experimental studies (Chiba et al., 2012; Voorhees et al., 2013). The drug treatment was started on 22nd day of the study and continued up to the 28th day. Drugs were administered by intraperitoneal (i.p.) route 1 h prior to the restraint stress exposure. Cognitive function was

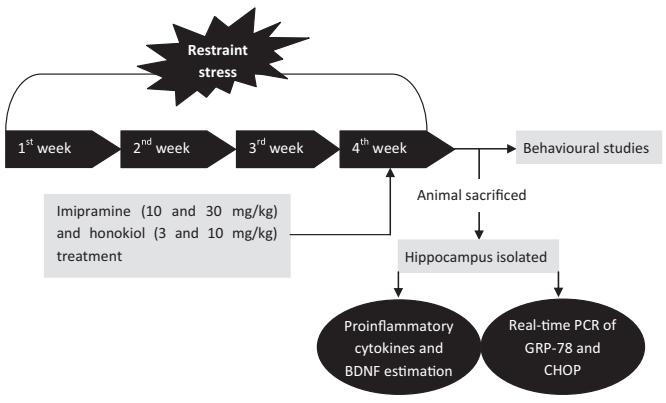

Fig. 1. Schematic representation of the study design for restraint stress and treatment schedule.