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Research Report

Farnesol attenuates lipopolysaccharide-induced neurodegeneration in Swiss albino mice by regulating intrinsic apoptotic cascade

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ABSTRACT

Neuronal apoptosis occurs as a sequel of oxidative stress associated with various neuropathies. In this study, we have investigated the protective effect of farnesol, a sequisterpene on lipopolysaccharide (LPS) induced neurodegeneration through modulation of intrinsic apoptotic cascade in the cortex and hippocampus of Swiss albino mice. Intraperitoneal (i.p.) injection of LPS (250 μg/kg b.wt. for 7 days) resulted in elevated levels of lipid peroxidation, protein carbonyls and 8-Hydroxydeoxyguanosine (8OHdG), with subsequent depletion in the antioxidant status and severe histological aberrations. These anomalies were accompanied by increased expressions of pro-apoptotic Bax, caspase-3 and p53 with decrease in anti-apoptotic Bcl-2. Farnesol treatment (100 mg/kg b.wt.) ameliorated LPS-induced oxidative stress by enhancing the antioxidant defense system as evident from the increased levels of SOD, CAT, GSH and GST and exhibited protected cellular morphology manifested from histopathological and nissl staining analyses. Farnesol treatment also reduced the expulsion of cytochrome c from mitochondria and downregulated caspase 3 activation as revealed by immunoblot analysis. Furthermore, farnesol treatment reduced the expression of Bax and antagonized LPS-induced decrease in anti-apoptotic Bcl-2. Results of this study show that farnesol exerts neuroprotective effect by regulating intrinsic apoptotic cascade through its antioxidant effect during LPS-induced neurodegeneration.

1. Introduction

Neuroinflammation and oxidative stress plays crucial role in the pathogenesis of chronic neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Huntington's disease and Multiple Sclerosis (Chong et al., 2005; Frank-Cannon et al., 2009). Microglia, the resident innate immune cells of the brain constitutes the prime defense against noxious agents (Saijo and Glass, 2011). Under normal physiological conditions, microglial cells monitor the brain parenchyma and maintain homeostasis (Nimmerjahn et al., 2005). However, aberrant activation due

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to diverse stimulus is neurotoxic and often detrimental leading to neurodegeneration (Streit et al., 2004).

Ligand of Toll-like receptor, lipopolysaccharide (LPS), a component of Gram-negative bacterial cell wall provides an experimentally potent challenge for the immune system by inducing expression of pro-inflammatory cytokines such as Interleukins (1β and 6) and TNF-α, from macrophages (Dobrovolskaia and Vogel, 2002). This peripheral inflammatory milieu can be relayed to the central nervous system through vagus nerve activation, circumventricular organs and brain endothelial cell activation (Akrout et al., 2009; Ching et al., 2007), activating resident microglia within the brain. Systemic administration of LPS activates microglia leading to neurodegeneration, suppression of neurogenesis and impairment of cognitive behavior in mice and rats (Lee et al., 2008; Okun et al., 2010; Qin et al., 2007).

Reactive oxygen species (ROS) is continuously produced in aerobic organisms as a byproduct of metabolism and during phagocytosis by macrophages including microglia. An imbalance in the redox homeostasis caused by ROS generation with a deficit in cellular antioxidant defense system renders the lipids, protein and DNA vulnerable to free radical attack culminating in oxidative stress that underlies multitudinous pathologies including neurodegeneration (Aruoma, 1998; Halliwell and Gutteridge, 1999). Studies on human brain and animal models of Alzhemeirs disease and Parkinsons disease shows an elevated levels of oxidative stress markers such as malondialdehyde (MDA), protein carbonyls and 8-hydroxydeoxyguanine (8OHdG) (Pugazhenthi et al., 2011; Gandhi and Abramov, 2012; Praticò, 2008; Dalfó et al., 2005). LPS was reported to deplete the levels of antioxidant enzymes and foster free radical generation by increasing brain MDA and reduction in GSH content (Kheir-Eldin et al., 2001; Sebai et al., 2009). Evidence shows that ROS mediated oxidative stress triggers apoptotic signal and subsequent neuronal death (Wang et al., 2013).

Apoptosis is a complex cascade of energy dependent biochemical and molecular events crucial in the development and maintenance of homeostasis of multicellular organisms (Smaili et al., 2003). Diverse cues of stimuli triggers intrinsic cell demise, resulting in cytochrome c release from mitochondria and subsequent activation of caspases. Caspase-3, a member of the cysteine proteases family plays a crucial role in dismantling of the cell during execution phase of apoptosis. The B-cell lymphoma 2 (Bcl-2)family of proteins regulates apoptosis through cytochrome c release from mitochondria negatively by anti-apoptotic (Bcl-2) and positively by pro-apoptotic (Bax, Bad) modulators (Elmore, 2007). The tumor suppressor protein p53 in turn mediates apoptosis by regulating the Bcl2 family of mitochondrial permeability regulators both by transcription dependent (Hoffman et al., 2002; Jeffers et al., 2003) and transcription independent (Wolff et al., 2008) mechanisms.

Farnesol is a 15-carbon, naturally occurring sesquiterpene present in essential oils of ambrette seeds and citronella. Farnesol was reported to posses significant antioxidant properties and protects renal cells against Fe-NTA induced oxidative stress (Jahangir et al., 2006). It has been demonstrated, that farnesol ameliorates inflammation and lung injury induced by intratracheal instillation of cigarette smoke extract in rats (Qamar and Sultana, 2008). Furthermore, chemopreventive effect of farnesol has been demonstrated in cancers of colon, liver and pancreas and skin (Rao et al., 2002; Ong et al., 2006; Burke et al., 2002; Chaudhary et al., 2009). In addition, a number of studies have demonstrated that farnesol inhibits cell proliferation and induces apoptosis in a spectrum of malignant cell types (Joo and Jetten, 2010; Scheper et al., 2008; Au-Yeung et al., 2008; Joo et al., 2007; Wiseman et al., 2007; Rioja et al., 2000; He et al., 1997). However, the neuroprotective role of farnesol has not been reported.

In the current study, we used aspects of LPS-induced peripheral inflammation to mimic chronic neurodegeneration to address the hypothesis that farnesol exhibits neuroprotective effect by ameliorating inflammation driven oxidative stress in the cortex and hippocampus of LPS administered Swiss albino mice. In this context, we have investigated the efficacy of farnesol in regulating intrinsic apoptotic cascade in terms of histopathological, biochemical aspects and expressions of Bax, Bcl-2, Caspase-3, Cytochrome C and p53 in the cortex and hippocampus of mice brain.

2. Results

2.1. *Farnesol enhances endogenous antioxidant status*

Table 2 represents the antioxidant status (SOD, CAT, GSH and GST) in the cortex and hippocampus of control and experimental groups of mice. A significant decrease ($p<0.05$) in the activities of antioxidants was observed in LPS-induced mice. These adverse changes in the antioxidant enzyme profile were reversed to near normal values in farnesol treated groups of mice. Though non-significant, an increase in antioxidant profile was observed in farnesol alone treated group when compared with control group of animals.

2.2. *Farnesol reduces MDA, protein carbonyl and 8-Hydroxydeoxyguanosine during LPS-induced oxidative stress*

The levels of malondialdehyde and protein carbonyl content (Fig. 1) were significantly increased ($p<0.05$) in the cortex and hippocampus of LPS-induced group as compared with control mice, whereas it was significantly ($p<0.05$) reduced in farnesol treated groups. However, no significant difference was observed between control and farnesol alone treated groups. Fig. 2 represents immunohistochemical analysis of 8-Hydroxydeoxyguanosine (8-OHdG) in the cortex and hippocampus [Cornu ammonis-1 (CA1)] of control and experimental group of animals. 8-OHdG is an integral marker of oxidative damage in cellular DNA. LPS-induced group of mice exhibited a significant increase ($p<0.05$) in 8-OHdG positive cells in the cortex and hippocampus (CA1) regions Fig. 2c and d. Farnesol treated group showed few 8-OHdG positive cells in the cortex and hippocampus Fig. 2e and f. No observable 8-OHdG positive cells were seen in control (Fig. 2a and b) and farnesol alone treated groups (Fig. 2g and h) respectively.