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Research article

Caffeic acid attenuates lipopolysaccharide-induced sickness behaviour and neuroinflammation in mice

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HIGHLIGHTS

- LPS induced sickness behaviour in mice, leading to reduced locomotor activity and increased immobility.
- CA treatment improved anti-oxidant defence and reversed the elevated TNF-α and IL-6.
- CA attenuated neuroinflammation mediated behavioural despair.

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ABSTRACT

Accumulating data links inflammation, oxidative stress and immune system in the pathophysiology of major depressive disorders. Sickness behaviour is a set of behavioural changes that develop during infection, eventually leading to decrease in mobility and depressed behaviour. Lipopolysaccharide (LPS) induces a depression-like state in animals that mimics sickness behaviour. Caffeic acid, a naturally occurring polyphenol, possesses antioxidant and anti-inflammatory properties. The present study was designed to explore the potential of caffeic acid against LPS-induced sickness behaviour in mice. Caffeic acid (30 mg/kg) and imipramine (15 mg/kg) were administered orally one hour prior to LPS (1.5 mg/kg) challenge. Behavioural assessment was carried out between 1 and 2 h and blood samples were collected at 3 h post-LPS injection. Additionally, cytokines (brain and serum) and brain oxidative stress markers were estimated. LPS increased the systemic and brain cytokine levels, altered the anti-oxidant defence and produced key signs of sickness behaviour in animals. Caffeic acid treatment significantly reduced the LPS-induced changes, including reduced expression of inflammatory markers in serum and whole brain. Caffeic acid also exerted an anti-oxidant effect, which was evident from the decreased levels of oxidative stress markers in whole brain. Our data suggests that caffeic acid can prevent the neuroinflammation-induced acute and probably the long term neurodegenerative changes.

1. Introduction

Sickness behaviour, which imitates depression, is manifested as reduced mobility, fatigue, cognitive impairment and inability to derive pleasure from otherwise enjoyable situations [1,2]. Sickness, which is a normal immune response of body to infection, accompanied by endocrine, autonomic and behavioural changes, is triggered by various soluble mediators. Although the brain is thought to be immune-privileged, there are multiple pathways by which these soluble mediators access the brain to cause neuroinflammation resulting in sickness behaviour [1,3]. There are overlapping mechanisms between depression and sickness behaviour [1,4], where sickness-induced peripherally released cytokines stimulate the central inflammatory pathways associated with depression [5]. Supportive evidence lies in the fact that patients undergoing cytokine therapy for cancer or viral diseases show depressed behaviour [6]. It is well established that systemic administration of LPS in experimental animals triggers the release

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of cytokines that leads to the activation of the innate immune system, thereby sending inflammatory signals through neuro-humoral pathways into the brain [5]. The intrinsic immune system of brain with microglia respond to these signals releasing pro-inflammatory cytokines leading to impaired serotoninergic and glutamatergic neurotransmission, finally resulting in sickness behaviour [6,7].

Naturally occurring dietary polyphenols through their pleiotropic mechanism exert multiple therapeutic properties [8]. One of the polyphenolic compounds, caffeic acid, is a catecholic compound, widely distributed in fruits, tea and wine [9]. Being one of the major representative members of the hydroxycinnamic acid class, caffeic acid is well known for its anti-oxidant [10], anti-tumour [11], anti-nociceptive [12] and anti-dementia properties [13]. In an earlier review, the authors have stated the neuroprotective properties of caffeic acid including anxiolytic effects [14]. Further, caffeic acid derivatives also possess anti-oxidant effects [15] and can prevent pentylenetetrazole-induced seizures in mice [16]. Based on the above evidences, the present study was aimed at exploring the neuroprotective effect of caffeic acid against sickness behaviour in mice.

2. Materials and methods

2.1. Animals

Male Swiss Albino mice (8–10 weeks, 20–30 g) were obtained from the inbred strains of Central Animal Research Facility (CARF), Manipal University. All the experiments were performed in accordance with the guidelines set out by the Institutional Animal Ethics Committee of Manipal University, following the CPCSEA guidelines and in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). The animals were housed in groups of 5, under controlled laboratory conditions, maintained on 12 h day and night cycle. Food and water were available *ad libitum*. Mice were handled individually every day for 2 days prior to the actual day of experiment.

2.2. Chemicals and reagents

All the chemicals used in this study were of analytical grade. Caffeic acid, lipopolysaccharides (*Escherichia coli* 0111:B4), 2-thiobarbituric acid, L-glutathione reduced, 5,5′-dithio-bis (2-nitrobenzoic acid) were purchased from Sigma-Aldrich Co. LLC (St Louis, MO, USA). Carboxymethyl cellulose, sodium dihydrogen phosphate anhydrous, disodium hydrogen phosphate anhydrous and trichloroacetic acid were purchased from Merck Millipore Corporation (Merck KGaA, Darmstadt, Germany).

2.3. Drug treatments

Animals were randomly assigned to four groups (n = 6), including, group I: saline (SAL); group II: LPS; group III: caffeic acid + LPS (CA + LPS) and group IV: imipramine + LPS (IMI + LPS). All the treatments were administered by oral route (*p.o.*), whereas LPS was given by interaperitoneal (*i.p.*) injection. Saline and LPS groups received vehicle, carboxymethyl cellulose (CMC, 0.25% w/v) at a dose of 10 mL/kg. CA + LPS and IMI + LPS groups were treated with caffeic acid (30 mg/kg) and imipramine (15 mg/kg) respectively. Sixty minutes after the treatments sickness behaviour was induced in all the animals except saline group using LPS (1.5 mg/kg). The selection of treatment doses was based on our preliminary studies with supporting literature evidence. Behavioural assays were performed within 1–2 h of LPS administration, and were video recorded. The recorded data were analysed by well-trained observers as well as by the blinded observers to reduce bias. The animals used for biochemical estimations were sacrificed by cervical dislocation 3 h post-LPS injections, serum and brain samples were rapidly collected and stored at −80 °C until further estimations. Brains were homogenised in ice cold phosphate buffer (0.1 M, pH 7.4) for estimation of anti-oxidant and cytokine levels.

2.4. Open field test

The open field test (OFT) was used to assess the effect of LPS and other treatment groups on exploratory behaviour of the animals. Locomotor activity (LMA) was assessed in mice individually placed into a clean, novel glass jar (30 cm × 30 cm × 60 cm). The open field was divided into nine virtual quadrants (10 cm × 10 cm) and LMA was measured by counting the number of line crossings, groomings and centre square entries over a 5 min period. The apparatus was cleaned with 70% ethanol between the experiments [17].

2.5. Forced swim test

The forced swim test (FST) was employed to study the effect of treatment groups on LPS-induced behavioural despair. The mice were forced to swim individually in a glass container (30 cm × 30 cm × 60 cm) containing water maintained at 22 ± 2 °C. The height of water level was adjusted to 15 cm. After an initial period of vigorous activity, the animals assume a type of immobile posture. A mouse is said to be immobile when it ceases struggling and makes minimal movement of limbs to keep the head above water. The total duration of test was 6 min with the immobility time recorded over the last 5 min of the study [18].

2.6. Tail suspension test

The tail suspension test (TST) was performed according to previously described methods [6]. Briefly, a small piece of medical adhesive tape was placed approximately 1–1.5 cm from the tip of the tail and the mice were hung individually for a period of 5 min at 15 cm away from the nearest surface. The tail climbing was prevented by placing plastic tubing around the tail prior to applying the tape. The duration of immobility was then measured during the final 4 min of the total 5 min of test.

2.7. Estimation of serum and brain cytokine levels

Serum and brain tumour necrosis factor-α (TNF-α) and serum interleukin-6 (IL-6) were estimated using murine TNF-α and IL-6 enzyme-linked immunosorbant assay (ELISA) kits (Novex®, Life Technologies, USA) respectively. The assays were performed as per manufacturer's instructions. The results were interpolated from the standard curve derived from TNF-α and IL-6 standards provided by the manufacturer.

2.8. Lipid peroxidation and reduced glutathione assay

Lipid peroxidation and reduced glutathione (GSH) in the brain homogenates were quantified according to the method of Janero [19] and Khan et al. [13], respectively. The amount of malondialdehyde (MDA) formed by the reaction with thiobarbituric acid was measured at 532 nm using UV–Vis spectrophotometer and expressed as nmoles/mg of protein. The amount of GSH was determined at 412 nm using a spectrophotometer and expressed as micromoles/mg of protein. Protein estimation was carried out using Pierce™ BCA Protein Assay Kit, as per manufacturer's instructions.