Pharmacological Reports 68 (2016) 764–772

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Possible neuroprotective mechanisms of clove oil against *icv*-colchicine induced cognitive dysfunction

Anil Kumar *, Archi Aggrawal, Raghavender Pottabathini, Arti Singh

Pharmacology Division, University Institute of Pharmaceutical Sciences, UGC Centre of Advanced Study, Panjab University, Chandigarh, India

ARTICLE INFO

Article history:
Received 11 December 2015
Received in revised form 6 March 2016
Accepted 7 March 2016
Available online 19 March 2016

Keywords:
Alzheimer's disease
Colchicine
Clove oil
Minocycline
Mitochondrial dysfunction

ABSTRACT

Background: Alzheimer's disease (AD), a common neurodegenerative disorder, recognized to be a major cause of dementia. The aim of the present study was to investigate the neuroprotective mechanisms of clove oil in intracerebroventricular *(icv)*-colchicine induced cognitive dysfunction in rats.
Methods: Single bilateral *icv*-colchicine (15 μg/5 μl) was administered, followed by drug treatment with clove oil (0.05 ml/kg and 0.1 ml/kg, *ip*), minocycline (25 and 50 mg/kg, *ip*) and their combinations for a period of 21 days. Various neurobehavioral parameters followed by biochemical, acetylcholinesterase (AChE) level and mitochondrial respiratory enzyme complexes (I-IV) were assessed.
Results: Colchicine *icv* administration significantly impaired cognitive performance in Morris water maze (MWM) causes oxidative stress, raised AChE level, caused neuroinflammation and mitochondrial dysfunction as compared to sham treatment. Treatment with clove oil (0.05 ml/kg and 0.1 ml/kg) and minocycline (25 and 50 mg/kg) alone significantly improved cognitive performance as evidenced by reduced transfer latency and increased time spent in target quadrant (TSTQ) in MWM task, reduced AChE activity, oxidative damage (reduced lipid peroxidation levels, nitrite level and restored glutathione levels) and restored mitochondrial respiratory enzyme complex (I-IV) activities as compared to *icv*-colchicine treatment. Further, combinations of clove oil (0.1 ml/kg) with minocycline (50 mg/kg) significantly modulate the neuroprotective effect of clove oil as compared to their effect alone.
Conclusion: The present study highlights that the major neuroprotective effect of clove oil due to its mitochondrial restoring and anti-oxidant properties along with a microglial inhibitory mechanism.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by progressive memory loss, cognitive impairment and personality defects accompanied by diffuse structural abnormalities in the brain [1]. The major pathological hallmarks of AD include beta amyloid (β-amyloid/Aβ) protein deposition, presence of neurofibrillary tangles and neurodegeneration especially of cholinergic neurons; neuro-inflammation, mitochondrial defects, and oxidative stress showing accumulation of reactive oxygen species and nitrogen species (ROS/RONS), energy metabolism defects [2]. Neuro-inflammation is an important feature of AD pathogenesis, consisting of the activation of both microglia and astrocytes [1]. Histological study revealed the presence of activated microglia and reactive astrocytes in and around extraneuronal Aβ plaques [2].

Colchicine can induce neurotoxicity and memory deficits by loss of cholinergic neurons, destruction of cholinergic pathways and decrease in cholinergic turnover mainly in the hippocampus area of the brain [3]. Intracerebroventricular (*icv*) colchicine treatment in rats developed an animal model of sporadic dementia of Alzheimer type due to inhibition of fast axoplasmic flow or direct toxic effect on cholinergic terminals [4].

Clove oil is known for its antioxidant and anti-inflammatory properties [5]. Eugenol, a major component of clove oil, has been reported to reduce colchicine-NMDA-Ca^{2+} influx induced neurotoxicity [6]. *Eugenia caryophyllata* was evaluated for its potential anti-inflammatory action on cyclooxygenase (COX)-2 and 15-LOX enzymes, 5-LOX-catalyzed lipid peroxidation [7], NF-κB pathway and interleukin (IL)-6 productions [8]. Eugenol has also been

* Corresponding author.
E-mail address: kumaruips@yahoo.com (A. Kumar).

http://dx.doi.org/10.1016/j.pharep.2016.03.005
1734-1140/

reported to possess anti-inflammatory activity by influencing microglial activation [6]. Clove oil has been reported to have a protective effect on NO production from neuroglial cells (C6 astrocyte cells) surrounding neurons contribute significantly to the pathogenesis of AD [5,6]. However, its mechanism of action is not clear.

Minocycline is reported to reduce the microglial activation and hence act as anti-inflammatory agent [9]. It effectively crosses the blood brain barrier and has widely reported neuroprotective effects [10,11]. But still the exact mechanism of neuroprotection and its effects on microglia are still unknown. Also, keeping this information in mind, intraperitoneal (*ip*) injection of minocycline is combines with the clove oil with an intention to get the superior therapeutic effect, if any, in *icv*-colchicine treated rats.

Therefore, the present study has been designed to investigate the possible neuroprotective mechanism of clove oil against *icv*-colchicine induced cognitive dysfunction.

Materials and methods

Animals

Male SD rats weighing 200–250 g were procured from the Central Animal House facility of Panjab University, Chandigarh. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. The rats were maintained under standard laboratory conditions with natural 12 h dark and light cycle and room temperature. They were allowed free access to standard dry diet and tap water *ad libitum*. All the behavioral procedures were carried out between 09:00 and 15:00 h. All procedures described were reviewed and approved by the Institutional Review Committee for the use of Human or Animal Subjects.

Surgery and intracerebroventricular (icv) administration of colchicine

Surgery was performed as per the procedure described by Javed [12]. Animals were anesthetized with chloral hydrate (350 mg/kg, *ip*) and positioned in a stereotaxic apparatus (Harvard, CA, USA). The head was positioned in a frame and a midline sagittal incision was made in the scalp. Two holes were drilled in the skull for the placement of a Hamilton syringe into the lateral cerebral ventricle. Co-ordinates for the *icv* injection were 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm beneath the cortical surface. The skin over the scalp was then sutured and neosporin powder, dental cement and fixer were applied. Artificial cerebrospinal fluid (ACSF; in mmol/l: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 dextrose) or 15 μg colchicine dissolved in ACSF were used to infuse into the brain of animals. Colchicine (5 μl) was injected using a Hamilton microsyringe into the stereotaxically positioned rat's brain. To promote the diffusion of drug, the microsyringe was left in place for a period of 2 min following injection.

Drugs and treatment schedule

Colchicine and clove oil were purchased from Sigma Aldrich (St. Louis, MO, USA) and minocycline from Wyeth Ltd., Mumbai, India. Colchicine solution was made fresh at the beginning of each experiment. Colchicine was prepared in ACSF such that a 15 μg dose was delivered in a 5 μl injection volume for *icv* administration. For *ip* administration, clove oil and minocycline were suspended in double distilled water along with a few drops of tween 80 to prepare solutions of the required doses of 0.05 and 0.1 ml/kg; 25 and 50 mg/kg, respectively once daily for 21 days. Animals were divided randomly based on their body weights into eight groups of 5-6 animals in each. The groups were as shown in Table 1 and the entire experimental protocol has been depicted in Fig. 1.

Measurement of body weight

The body weights of the animals were recorded before *icv* administration of colchicine (day −1) and on the last day of the study (day 21) [13]. Percentage change in body weight was calculated as: Percentage change in body weight = Body weight [(Day 21 - Day 1)/Day 1] × 100.

Behavioral assessments

Assessment of locomotor activity

Spontaneous locomotor activity was assessed on day 0 before surgery, 7, 14 and 21 of colchicine administration. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light sensitive photocells using a digital photoactometer (IMCORP, Ambala, India) and values expressed as counts/5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room [9,13].

Assessment of memory performance

Spatial navigation task. It is performed through the Morris water maze task.

Morris water-maze (MWM) task. MWM task is most commonly used to test memory [14], consisting of a large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1 °C). The tank was divided into four equal quadrants and a submerged platform (10 cm × 10 cm), placed 1 cm below the surface of water in the middle of the target quadrant. The position of the platform was kept unaltered throughout the training session. This test includes acquisition phase and probe trial phase. During the acquisition phase, task was carried out for four consecutive days (17th to 20th day) where animals received four consecutive daily training trials, each at an interval of 30 min approximately. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition or spatial

Table 1
Treatment groups.

S. no.	Group (mg/kg)	Drug treatment
1	Naive	No treatment
2	Sham	Surgery performed and only vehicle (ACSF) administered
3	Control	Single bilateral colchicine (15 μg/5 μl) administration
4	CO (0.05)	Clove oil (0.05 ml/kg, *ip*) + colchicine (15 μg/5 μl) for a period of 21 days
5	CO (0.1)	Clove oil (0.1 ml/kg, *ip*) + colchicine (15 μg/5 μl) for a period of 21 days
6	Mino (25)	Minocycline (25 mg/kg, *ip*) + colchicine (15 μg/5 μl) for a period of 21 days
7	Mino (50)	Minocycline (50 mg/kg, *ip*) + colchicine (15 μg/5 μl) for a period of 21 days
8	CO (0.1) + Mino (50)	Clove oil (0.1 ml/kg, *ip*) + minocycline (50 mg/kg, *ip*) + colchicine (15 μg/5 μl) for a period of 21 days