Biomedicine & Pharmacotherapy 81 (2016) 56–62

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Caffeic acid attenuates oxidative stress, learning and memory deficit in intra-cerebroventricular streptozotocin induced experimental dementia in rats

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ARTICLE INFO

Article history:
Received 5 March 2016
Received in revised form 15 March 2016
Accepted 15 March 2016

Keywords:
Caffeic acid
Cognitive dysfunction
Oxidative stress
Sporadic dementia
Streptozotocin

ABSTRACT

Oxidative stress has been implicated in cognitive decline as seen during normal aging and in sporadic Alzheimer's disease (AD). Caffeic acid, a polyphenolic compound, has been reported to possess potent antioxidant and neuroprotective properties. The role of caffeic acid in experimental dementia is not fully understood. Thus the present study was designed to investigate the therapeutic potential of caffeic acid in streptozotocin (STZ)-induced experimental dementia of Alzheimer's type in rats. Streptozotocin (STZ) was administered intracerebroventrically (ICV) on day 1 and 3 (3 mg/kg, ICV bilaterally) in Wistar rats. Caffeic acid was administered (10, 20 and 40 mg/kg/day p.o.) 1 h following STZ infusion upto 21st day. Morris water maze and object recognition task were used to assess learning and memory in rats. Terminally, acetylcholinesterase (AChE) activity and the levels of oxido-nitrosative stress markers were determined in cortical and hippocampal brain regions of rats. STZ produced significant ($p < 0.001$) learning and memory impairment, oxido-nitrosative stress and cholinergic deficit in rats. Whereas, caffeic acid treatment significantly ($p < 0.001$) and dose dependently attenuated STZ induced behavioral and biochemical abnormalities in rats. The observed cognitive improvement following caffeic acid in STZ treated rats may be due to its antioxidant activity and restoration of cholinergic functions. Our results suggest the therapeutic potential of caffeic acid in cognitive disorders such as AD.

1. Introduction

The term 'dementia' refers to memory impairment and loss of other intellectual abilities which interfere with normal daily activities. The prevalence of dementia is increasing worldwide and became a major public issue with the increasing elderly population [21]. It has been well documented that memory and cognitive impairment is associated with both physiological aging and central nervous system pathological conditions, including stroke, Parkinson's disease (PD) and Alzheimer's disease (AD) etc. Among them Alzheimer disease is most common form of dementia.

Many studies have revealed that free radical generation cause oxidative stress and damage to macromolecules (lipid, protein and nucleic acids, etc.) which has been considered as an important factor in the acceleration of aging and age-related neurodegenerative disorders associated with dementia [6]. Currently available drug therapy provide modest improvement on cognitive and global measures relevant to dementia [36]. Therefore, there is a great need for drugs that counteract the processes involved in ageing more specifically the decline in memory and cognitive functions.

Recently, interest has been focused on a group of phytochemicals such as polyphenols, which are found to improve learning and memory [15,34]. Polyphenols can be broadly divided into two categories, flavonoids and non-flavonoid polyphenols. Caffeic acid (3,4-dihydroxycinnamic acid) is a well-known nonflavonoid phenolic phytochemical present in fruits, tea and wine [7]. Even though caffeic acid is a phenolic compound especially abundant in coffee, it is chemically unrelated to caffeine [16]. Caffeic acid has been demonstrated to possess a broad spectrum of pharmacological

Abbreviations: 6-OHDA, 6-hydroxydopamine; ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; ANOVA, analysis of Variance; Aβ, amyloid-beta; BSA, bovine serum albumin; Caffeic acid, 3,4-dihydroxycinnamic acid; DMSO, dimethylsulfoxide; DNPH, 2,4-dinitrophenylhydrazine; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GSH, reduced glutathione; IAEC, Institutional Animal Ethics Committee; ICV, intracerebroventrically; INSA, Indian National Science Academy; MDA, malondialdehyde; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MWM, Morris water maze; ORT, object recognition task; p.o., per oral; PD, Parkinson's disease; PMS, post-mitochondrial supernatant; ROS, reactive oxygen species; SAD, sporadic Alzheimer's disease; STZ, streptozotocin; TCA, tricarboxylic acid.

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http://dx.doi.org/10.1016/j.biopha.2016.03.017
0753-3322/

activities including anti-inflammatory, antioxidant and Immunomodulatory effects [2]. In recent years various studies have demonstrated the neuroprotective potential of caffeic acid against stroke and hydrogen peroxide-induced oxidative brain damage and acrolein, MPTP and 6-OHDA induced toxicities [28]. Caffeicacid has been demonstrated to protect amyloid-β- induced neurotoxicity in PC12 cells [35]. Moreover, caffeic acidhas also been reported to enhance brain acetylcholine levels and improvelearning and memory [40]. These evidences suggest that the caffeic acid could proveto be useful candidate molecule in the therapeutic management of cognitive disorders such as Alzheimer's disease. However, the neuroprotective potential of caffeic acid is least investigated. Intracerebroventricular administration of streptozotocin is well known to produce behavioral, biochemical and neuropathological changes similar to that seen in sporadic Alzheimer's disease (SAD) and considered as an appropriate animal model of SAD [22]. Thus, in the present study, we have investigated the neuroprotective potential of caffeic acid against intracerebroventricular streptozotocin-induced neurocognitive deficit and oxidative stress in rats.

2. Method

2.1. Animals

The experiments were carried out in male Wistar rats (250–300 g) obtained from Central Animal House of I.S.F. College of Pharmacy, Moga, Punjab (India). They were kept in polyacrylic cages (4/cage) and maintained under standard husbandry conditions (room temperature 22 ± 1 °C and relative humidity of 60%) with 12 h light/dark cycle (lights on at 8 AM). The food in the form of dry pallets and water were made available *ad libitum*. All behavioral experiments were carried out between 9 PM and 4 AM as night time is the active phase of rodents. The protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) and the animal experiments were carried out in accordance with the Indian National Science Academy (INSA) guidelines for use and care of animals. The research was conducted in accordance with internationally accepted principles for laboratory animal use and care.

2.2. Drugs and chemicals

Streptozotocin was purchased from Sigma-Aldrich, USA. Caffeic acid was purchased from Cayman Chemicals, USA. The purity of caffeic acid was ≥97%. Category no. 70602; Lot number: 184033-188627. All other chemicals used in the study were of analytical grade. Solutions of the drugs and chemicals were always prepared afresh before use.

2.3. Experimental procedure

The rats were anesthetized with ketamine (100 mg/kg, i.p) and xylazine (5 mg/kg, i.p). Rat was fixed in a stereotactic apparatus and the skull was exposed after midline sagittal incision. Two burr holes were drilled through the skull and cannulas were placed bilaterally into the lateral cerebral ventricles using following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to sagittal suture; 3.6 mm ventral from the surface of the brain [27] and then fixed with dental cement. Animals were divided into five groups and each group comprised of eight (8) animals. Group1: served as double vehicle control, received citrate buffer (pH 4.4) as a vehicle for STZ given ICV in a volume of 5 μl bilaterally on day 1 and 3; and 2% dimethylsulfoxide (DMSO) diluted with saline (as a vehicle for caffeic acid) was administered (5 ml/ kg)orally for 21days. Group 2: Rats were infused (1 μl/min) with ICV-STZ (3 mg/kg) dissolved in citrate buffer (pH 4.4) in a volume of 5 μl in each lateral ventricle on day 1 and 3. Group 3, 4 and 5: received caffeic acid at doses of 10, 20 and 40 mg/kg p.o., respectively and group 6 received donepezil (Dnp) 5 mg/kg as a standard treatment group, 1 h following 1st STZ infusion on day 1 and continued once daily for a period of 21 days. The treatment schedule and the intervals for estimation of various parameters are presented in Fig. 1. The doses of caffeic acid were selected on the basis of earlier report demonstrating significant antioxidant and neuroprotective potential in rats [2].

2.4. Behavioral assessment

2.4.1. Object recognition test (ORT)

The ORT was performed as described elsewhere [13] with minor modifications. The ORT was performed in a wooden open box apparatus (80 × 60 × 40 cm). The objects to be discriminated were of two different shapes (triangle and cylindrical), made up of painted wood, around 10 cm in height and heavy (so that cannot be displaced by the animals during test). In addition, these objects had no genuine significance for rats and had never been associated with reinforcement. The day before testing, rats were allowed to explore the apparatus for a 15 min session of habituation. Twenty four hours later, the first 3 min sample trial test (T1), with two similar objects of shape and color (termed as sample objects F01 and FO1) presented at the corners of the box, was started. Following T1, all the rats were placed back in their home cage and a delay of 60 min was given as inter-trial interval for T2. In the second 3-min choice trial (T2), one of the objects presented in T1 was replaced by a novel object (NO). All the combinations and locations of the objects were used in a balanced manner to reduce potential bias due to preferences for particular locations or objects. To avoid the presence of olfactory trails, the apparatus and the objects were cleaned thoroughly after each trial. Exploration was considered as directing the nose to the objects at a distance ≤2 cm to the objects and/or touching it with the nose. The times spent by rats in exploring two objects in T1 and T2 were recorded separately. A series of variables was then calculated: total time spent in exploring two identical objects in T1. The discrimination between the familiar and the novel object during T2 was measured by comparing the time spent in exploring the familiar object with that

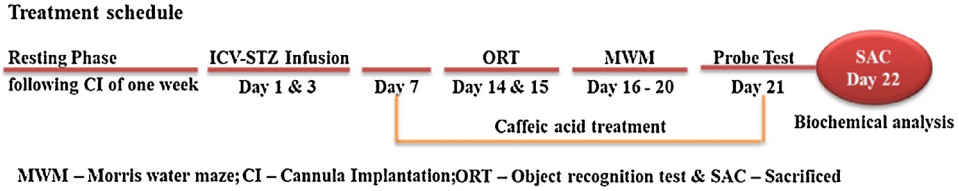

Fig. 1. Treatment schedule.