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Caffeic acid phenethyl ester rescued streptozotocin-induced memory loss through PI3-kinase dependent pathway

Manish Kumar[a,b], Nitin Bansal[b,*]

[a] PhD Research Scholar, IKG Punjab Technical University, Kapurthala, Punjab, 144603, India
[b] Department of Pharmacology, ASBASJSM College of Pharmacy, Bela, Ropar, 140111, India

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ABSTRACT

The present study was undertaken to elucidate the role of PI3-kinase signaling in memory enhancing potential of caffeic acid phenethyl ester (CAPE) against cognitive defects in rats after centrally administered streptozotocin as a model of Alzheimer's disease. The Morris water maze and elevated plus maze paradigms showed profound loss of memory in adult Wistar rats (180–200 g) injected with streptozotocin (3 mg/kg) bilaterally (STZ-ICV) on day 1 and 3. Intraperitoneal administration of CAPE (6 mg/kg, *i.p.*, 28 days) attenuated STZ-ICV triggered memory loss in rats. Treatment with PI3-kinase inhibitor (wortmannin, 5 µg/rat, ICV) or NOS blocker (L-NAME, 20 mg/kg, *i.p.*, 28 days) interfered with memory restorative function of CAPE in STZ treated rats. In biochemical analysis markers of oxidative stress (TBARS, GSH, SOD, CAT), nitrite, AChE, TNF-α, eNOS and NFκB were measured in brain of rats on day 28. Interestingly, L-Arginine (100 mg/kg, *i.p.*, 28 days) group exhibited moderate ($p > 0.05$) decline in memory functions. The brain oxidative stress, TNF-α, AChE activity and NFκB levels were elevated, and eNOS level was lowered by STZ-ICV treatment. Administration of CAPE lowered oxidative stress, AChE, nitrite and TNF-α levels in brain of rats. The eNOS level was enhanced and NFκB level was decreased by CAPE in STZ treated rats. Wortmannin injection elevated the brain oxidative stress, AChE activity and TNF-α levels, and decreased the nitrite, eNOS and NFκB level. Rise of brain oxidative stress parameters, AChE activity, TNF-α, eNOS and NFκB levels, and decline in brain nitrite content was observed in L-NAME treated group. L-Arginine administration showed modest effects ($p > 0.05$) on oxidative stress parameters. Brain nitrite content was enhanced although eNOS, NFκB levels, and AChE activity was decimated by L-Arginine treatment. It can be concluded that PI3-kinase mediated nitric oxide facilitation is an essential feature of CAPE action in STZ-ICV treated rats.

1. Introduction

Over the years, considerable research for new therapeutically active molecules has witnessed significant increase in life-expectancy of human species although with concomitant rise in age associated disorders such as Alzheimer's disease (AD). Several lines of evidences indicate hastened biogenesis of free radicals in brain and release of pro-inflammatory chemokines in primordial stages of AD neuropathology [1]. Coexistence of oxidative stress and insulin dysfunction exacerbate the amyloidogenic processing of brain amyloid precursor protein (APP), β-secretase (BACE) activity, hyperphosphorylated tau (pTau) and subsequent neurodegeneration in age associated AD pathology [2]. Recently, phosphoinositide (PI) mediated lipid signaling has provided a link between AD etiologic factors such as ageing, brain glucose metabolism, oxidative stress, neuroinflammation and ensuing cognitive impairment [3,4]. Phosphoinositide-3-kinase (PI3-kinase) comprises of cytosolic or membrane associated signal-transducer enzymes that phosphorylate phosphatidylinositol (e.g. PIP_2) to generate various PIs (e.g. PIP_3) entailed for activation of Akt through pleckstrin homology (PH) domain [5]. PI3-kinase acts downstream of various major receptors such as tyrosine kinase and G-protein coupled receptors, and is a major cell survival pathway that regulates nitric oxide signaling (e.g. eNOS), synaptic function, glucose metabolism (e.g. GSK-3), transcription factors (e.g. NFκB), cell cycle progression (e.g. CDK-2, cyclin-A), cell migration, proliferation and apoptosis (e.g. p53, Bcl-2, BAD) in response to several extracellular signals including insulin, growth factors (e.g. IGF, BDNF) and hormones. The anti-apoptotic and pro-survival functions render PI3-kinase activity highly desirable in neuropathology of AD. Altered PI3-kinase activity in brain is related with spurt in free radicals, pTau, APP and BACE-1 hyperactivity [6,7,8]. Studies supporting the link between Akt signaling and AD have shown that PI3-kinase activation confer neuroprotection and improves

* Corresponding author at: Department of Pharmacology, ASBASJSM College of Pharmacy, Bela, Ropar, Punjab 140111, India.
E-mail addresses: manishkumar@swiftcollegeedu.in (M. Kumar), nitindsp@rediffmail.com (N. Bansal).

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memory [9].

Caffeic acid phenethyl ester (CAPE) is the bioactive component of the propolis derived from the hives of honeybees. It is a highly lipophilic polyphenol with high volume of distribution and easy access to brain [10]. The pro-inflammatory molecules such as cycloxygenase, lipoxygenase and NFκB are key targets of CAPE that prompted its usage against diverse inflammatory conditions [11]. The upregulation of Nrf2/ARE signaling by phenylpropanoid scaffold imparts potent antioxidative property to CAPE [12]. The neuroprotective potential of caffeic acid phenethyl ester (CAPE) against several neurotoxins has been demonstrated by *in vivo* and *in vitro* studies. A decrease in β-secretase activity and increase in α-secretase activity is observed in hippocampal cell culture indicating the cytoprotective activity of CAPE [13]. CAPE is associated with insulin induced enhancement of glucose metabolism, cessation of expression of pro-apoptotic factors and neuroapoptosis through activation of PI3-kinase pathway [14,15]. Resurrection of neurovascular integrity against traumatic brain injury and attenuation of cerebral vasospasm in rodents by CAPE is indicative of involvement of PI3-kinase signaling [16,17]. Previously our lab reported memory enhancing potential of CAPE in rats against streptozotocin (STZ) induced AD type dementia [18]. However, the putative molecular mechanism of neuroprotective action of CAPE is still elusive. STZ is a diabetogenic drug widely used to precipitate AD like neurodegenerative changes through intracerebroventricular (ICV) administration in rodents. The present study delineates role of PI3-kinase signaling in resurrection of memory in rats by CAPE in intracerebroventricle administered streptozotocin (STZ-ICV) model of AD.

2. Materials and methods

2.1. Experimental animals

The research protocol of this study was approved by Institutional Animal Ethics Committee of the institute and Wistar rats (180–220 g, either sex) were procured from Central Animal Facility, All India Institute of Medical Sciences, New Delhi; and were nurtured at Central Animal Facility of the institute under standard laboratory conditions with controlled temperature (23 ± 2 °C), humidity (40 ± 10%) and natural light-dark cycle (12 h each). The animals were housed in polyacrylic cages in group of three per cage (44 × 29 × 16 cm^3), and nourished with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The experiments were carried out between 09:00 and 18:00 h. The care of laboratory animals was done following the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India.

2.2. Drugs and chemicals

Caffeic acid phenethyl ester, acetylthiocholine iodide, wortmannin, L-NAME from Sigma-Aldrich; streptozotocin, nitrobluetetrazolium chloride, sodium azide, dimethylsulfoxide from Sisco Research Laboratories Pvt Ltd., Mumbai; L-Arginine, riboflavin, 5,5′-Dithiobis(2-nitrobenzoic acid), chloral hydrate, formalin, Harris hematoxylin, eosin from Himedia Laboratories, Mumbai; sodium nitrite, hydrogen peroxide, triton-X-100, N-1-Napthylethylenediamine dihydrochloride, thiobarbituric acid, trichloroacetic acid, sodium dodecyl sulphate, sodium cyanide, Folin-Ciocalteu Phenol reagent from Loba Chemie, Mumbai; sulphanilamide (Spectro Chem); n-butanol (Merck); TNF-α ELISA kit (Krishgen, Mumbai); eNOS ELISA kit (KinesisDX, California); NFκB ELISA kit (KinesisDX, California) were used. Artificial cerebrospinal fluid (aCSF) was prepared as following: 147 mM NaCl (0.0859 g), 2.9 mM KCl (0.00216 g), 1.6 mM $MgCl_2$ (0.00152 g), 1.7 mM $CaCl_2$ (0.00249 g), 2.2 mM dextrose (0.00396 g) in 10 ml of water for injection (pH 7.3) [18].

2.3. Surgery of rat brain

The animals were acclimatized to laboratory conditions two weeks prior to the surgical procedure. Chloral hydrate (300 mg/kg, *i.p.*) was injected and animal was rendered unresponsive before continuing surgery. Depth of anesthesia was determined by lack of pedal pain in tail/toe pinch-response method and loss of corneal reflex/blinking when air is blown into eyes. During the surgery a heating pad was used to maintain the body temperature at 37 ± 0.5 °C. The body of anesthetized rat was placed on a warm pad and head was positioned in the frame of stereotaxic apparatus (INCO, Ambala, India). The partially shaved scalp was wiped with 70% ethyl alcohol swab before incision. A middle sagittal incision was made in the scalp, skin was retracted and skull was exposed. A burr hole was drilled through the parietal bone of skull by using stereotaxic coordinates: antero-posterior from bregma = -0.8 mm, mediolateral from mid-sagittal suture = ± 1.5 mm, dorsoventral from the skull = ± 3.6 mm; in order to access a randomly selected lateral cerebral ventricle [19]. After drug administration the holes were repaired with dental cement and skin was sutured. Neosporin® was applied for some days to prevent contamination. To avoid sepsis Reflin® (cephazolin sodium, Ranbaxy) was injected (30 mg/kg, *i.p.*) once postoperatively. Postsurgical hypothermia was prevented by keeping the rats warm. Each rat was housed in individual cage (30 × 23 × 14 cm^3) for 7 days after surgery, and provided free access to food and water.

2.4. Drug treatments

The animals were randomly divided into six different groups having six animals in each group. Control group (Sham-treated) received ICV-vehicle (10 µl) only; STZ group (STZ-ICV) rats were given streptozotocin (3 mg/kg, 10 µl) alone; CAPE + STZ group (CAPE + STZ) was administered CAPE (6 mg/kg, *i.p.*) for 28 days and STZ-ICV (3 mg/kg, 10 µl); Wortmannin group (CAPE + STZ + Wort) received CAPE (6 mg/kg, *i.p.*) for 28 days, and STZ (3 mg/kg, 8 µl) and wortmannin (5 µg/rat in two divided doses 2.5 µg in 2 µl); L-NAME group (CAPE + STZ + L-NAME) rats received CAPE (6 mg/kg, *i.p.*) and L-NAME (20 mg/kg, *i.p.*) for 28 days, and STZ-ICV (3 mg/kg, 10 µl); L-Arginine group (CAPE + STZ + L-Arg) rats were administered with CAPE (6 mg/kg, *i.p.*) and L-Arginine (100 mg/kg, *i.p.*) for 28 days, and STZ-ICV (3 mg/kg, 10 µl).

Streptozotocin (3 mg/kg *b.w.*) was administered bilaterally in rats on day 1 and 3 to induce cognitive deficits of AD type [20]. A 3 mg/kg dose of STZ having appropriate concentration was freshly prepared using 5% dimethylsulfoxide (DMSO) in aCSF as ICV-vehicle [21]. On first day the rats received streptozotocin (3 mg/kg) in lateral cerebral ventricle except control group rats. In Wortmannin group ICV administration of STZ (3 mg/kg, 8 µl) was followed by wortmannin (2.5 µg, 2 µl ICV-vehicle). Sham rats received same volume of ICV-vehicle only. ICV treatment was repeated in other lateral ventricle once after 48 h. Central administration of wortmannin in dose ranges 0.5 ng-2.5 µg per rat irreversibly inhibits the PI3-kinase activity (IC_{50} value 4–9 nM for PI3-kinase subunits) [22] and phosphorylation of Akt [21,23,24]. The total ICV injection volume was limited to 10 µl per day. Hamilton® microsyringe was switched between different administrations without disturbing the microneedle. After injection the microneedle (28 gauge) was not displaced for 5 min to prevent pressure backflow (reflux) of drug solution out of ventricle along the injection track and thereby enhance diffusivity of drug in CSF. Rate of injection was maintained at 1 µl/min.

CAPE (6 mg/kg) was dissolved in sterile DMSO diluted with isotonic saline (1:5) and was injected intraperitoneally one hour before STZ administration and continued for 28 days daily [18] (Fig. 1). Administration of $N^{(G)}$-nitro-L-arginine methyl ester (20 mg/kg, *i.p.*) and L-Arginine (100 mg/kg, *i.p.*) solutions in normal saline was initiated on day 1 after STZ treatment which continued up to day 28 [25]. It is