European Journal of Pharmacology 659 (2011) 177–186

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Behavioural Pharmacology

Modulation of nitrergic pathway by sesamol prevents cognitive deficits and associated biochemical alterations in intracerebroventricular streptozotocin administered rats

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ARTICLE INFO

Article history:
Received 22 September 2010
Received in revised form 25 February 2011
Accepted 21 March 2011
Available online 2 April 2011

Keywords:
Alzheimer's disease
Intracerebroventricular streptozotocin
L-arginine
L-NAME (N(G)-nitro-L-arginine methyl ester)
Sesamol
TNF-α (tumor necrosis factor-alpha)

ABSTRACT

Alzheimer's disease is a neurodegenerative disorder characterized by progressive cognitive decline and widespread loss of neurons and their synapses in the cerebral cortex and hippocampus. Increasing evidence indicates that factors such as oxidative–nitrergic stress, glutathione depletion, impaired protein metabolism and cholinergic deficit can interact in a vicious cycle, which is central to Alzheimer's disease pathogenesis. Intracerebroventricular (i.c.v.) streptozotocin induced-cognitive impairment has been widely used as an experimental paradigm to study Alzheimer's disease. In the present study, i.c.v. streptozotocin produced significant cognitive deficits as measured in Morris water maze and elevated plus maze task coupled with increased serum TNF-α levels and marked rise in brain acetylcholinesterase and oxidative–nitrergic stress in female Wistar rats. Sesamol (5-hydroxy-1,3-benzodioxole or 3,4-methylenedioxyphenol), a potent anti-oxidant and anti-inflammatory molecule markedly improved cognitive impairment, reduced acetylcholin-esterase activity, TNF-α levels and attenuated oxidative–nitrergic stress in brain of i.c.v.-streptozotocin treated rats. Administration of L-arginine (125 mg/kg i.p), a nitric oxide donor, alone to i.c.v.-streptozotocin treated rats accentuated behavioral and biochemical deficits and also abolished the protective effect of sesamol (8 mg/kg). L-NAME (10 mg/kg i.p.), a non-specific NOS inhibitor significantly restored all the behavioral and biochemical indices in i.c.v.-streptozotocin rats. Moreover, combination of L-NAME with sub-effective dose of sesamol (4 mg/kg) potentiated its protective effect. Our findings demonstrate the effectiveness of sesamol in preventing intracerebroventricular streptozotocin-induced cognitive deficits by modulating nitrergic signaling and oxido-inflammatory cascade.

1. Introduction

Alzheimer's disease is the most common type of dementia in Western societies and has profound economic and social impact. It accounts for 50% of dementia cases all around the globe (Areosa and Sherriff, 2006). Alzheimer's disease is characterized by marked atrophy of cerebral neocortex, hippocampus and loss of cortical and sub-cortical neurons (Vance et al., 2005). Neuroscientists all around the world are trying their best to develop a sure-shot remedy for Alzheimer's disease and related dementia. Current strategies are mainly focused on two aspects; one is to develop agents, which improve cognitive deficits of Alzheimer's disease and second to find appropriate experimental models for screening of such agents (Vance et al., 2005). The intracerebroventricular streptozotocin (i.c.v. strep-tozotocin) injected rat has been described as an appropriate animal model for sporadic dementia characterized by progressive deteriora-tion of memory and cerebral glucose and energy metabolism, along with oxidative stress (Lannert and Hoyer, 1998; Sharma and Gupta, 2001a; Sonkusare et al., 2005).

Nitric oxide mediated nitrergic signaling, an important bioregula-tory signaling in the nervous, immune and cardiovascular system, plays a pivotal role in brain homeostasis. The involvement of nitric oxide in a number of neurological disorders is well established. Researchers have begun to recognize and to explore the putative link between nitric oxide and Alzheimer's disease (Dorheim et al., 1994; Norris et al., 1996). Although existing evidence regarding their association is not abundant, emerging data are showing Alzheimer's disease-related changes in the nitric oxide synthase system, and it appears that nitric oxide could be related to many of the pathological mechanisms of the disease. The ability of nitric oxide to exert cellular damage due to its reactive oxidative properties is perhaps the primary neurotoxic mechanism. The presence of a stimulus that leads to the overproduction of nitric oxide will likely cause neuronal damage (Law et al., 2001).

Since oxidative damage is implicated in the etiology of neurolog-ical complications, treatment with antioxidants has been used as a

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0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.03.026

therapeutic approach in various types of neurodegenerative diseases (Ahmad et al., 2005; Ansari et al., 2004). Sesamol (5-hydroxy-1,3-benzodioxole or 3,4-methylenedioxyphenol) is the major constituent of sesame seed oil *Sesamum indicum* L and is a powerful antioxidant that inhibits ultra violet and Fe3+/ascorbate-induced lipid peroxidation in rat brain (Prasad et al., 2005). Sesamol also possess neuroprotective (Hou et al., 2006), hepatoprotective (Hsu et al., 2006), anti-inflammatory (Hou et al., 2006), chemo-preventive (Prasad et al., 2005) and anti-aging properties (Sharma and Kaur, 2006).

With this background, the present study was designed to investigate the possible protective effect of sesamol on streptozotocin-induced neurotoxicity and to explore the possible involvement of nitrergic signaling. The functional interaction of sesamol with nitrergic signaling was investigated using nitric oxide precursor, L-arginine, and non selective nitric oxide synthase inhibitor, N(G)-nitro-L-arginine methyl ester (L-NAME).

2. Material and methods

2.1. Animals care

Adult female Wistar rats (250–300 g) bred in Central Animal House facility of Panjab University were used with 5–8 animals in each group. The female rats were used as they are more prone to the cognitive impairment (Roof and Stein, 1999). The rats were housed in polyacrylic cages [38×23×10 cm] and maintained under standard laboratory conditions with natural dark and light (12:12 h) cycle and had free access to food (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Animals were acclimatized to laboratory conditions before all the behavioral tests. All experiments were carried out between 0900 and 1700 h and performed in accordance with the Guidelines of EC Directive 86/609/EEC for animal experiments. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Panjab University and performed in accordance with the guidelines of the Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.

2.2. Drugs and treatment

Sesamol, L-arginine, L-NAME and streptozotocin were purchased from Sigma Aldrich, St. Louis, MO, USA. Sesamol, L-arginine and L-NAME were dissolved in double distilled water while streptozotocin was dissolved in artificial cerebrospinal fluid (aCSF) (2.9 mM KCl, 147 mMNaCl, 1.7 mM$CaCl_2$, 1.6 mM $MgCl_2$, and 2.2 mM D-glucose). The doses of sesamol and nitric oxide modulators were selected according to the previous studies conducted in our laboratory (Kuhad and Chopra, 2008; Chander et al., 2005). All drug solutions were freshly prepared immediately prior to injection. Sesamol was administered per orally whereas L-arginine and L-NAME were injected intraperitoneally.

2.3. Surgical procedures: intracerebroventricular injection of streptozotocin

Intracerebroventricular injection of streptozotocin was performed according to the procedure of Sonkusare et al. (2005). Rats were anesthetized with thiopentone (Neon Laboratories, India, 45 mg/kg, i.p.). The scalp was shaved, cleaned and cut to expose the skull. The head was positioned in a stereotaxic frame and a midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles by using the following coordinates: 0.8 mm posterior to bregma; 1.5 mm lateral to sagittal suture and 3.6 mm beneath the surface of the brain (Sharma and Gupta, 2002). Streptozotocin (3 mg/kg, intracerebroventricular) was injected bilaterally in two divided doses on first and third day making the dose of 1.5 mg/kg each day. The concentration of streptozotocin in aCSF was adjusted so as to deliver 10 µl of the solution. Control animals received intracerebroventricular injection of the same volume of aCSF on the first and third day. The skin was sutured after second injection followed by daily application of antiseptic powder (Neosporin). Postoperatively, the rats were fed with oral glucose and normal pellet diet for 4 days, followed by normal pellet diet alone.

2.4. Experimental design

Rats were randomly divided into ten different groups containing 5–8 animals in each group viz Group 1: control animals received an equivalent volume of vehicle for streptozotocin i.e. artificial CSF (aCSF) on day 1 and day 3; Groups 2 and 3: animals received intracerebroventricular injections of aCSF on day 1 and day 3 along with L-arginine (125 mg/kg; intraperitoneal) and L-NAME (10 mg/kg; intraperitoneal) respectively for 21 days; Group 4: animals received intracerebroventricular injection of streptozotocin 1.5 mg/kg on each day 1 and day 3; Groups 5, 6 and 7: i.c.v.-streptozotocin treated rats administered sesamol (4 mg/kg), L-NAME (10 mg/kg; intraperitoneal) and L-NAME (10 mg/kg; intraperitoneal) + sesamol (4 mg/kg) respectively for 21 days; Groups 8, 9 and 10: i.c.v.-streptozotocin treated rats received sesamol (8 mg/kg; oral gavage), L-arginine (125 mg/kg; intraperitoneal) and L-arginine (125 mg/kg; intraperitoneal) + sesamol (8 mg/kg; oral gavage) respectively for 21 days.

From the preliminary data of the present study, we have selected two doses of sesamol (4 and 8 mg/kg) for further drug interaction studies with nitric oxide modulators. The possible participation of the nitric oxide signaling in the neuroprotective effect of sesamol was investigated. Mice were pretreated with L-arginine, a precursor of nitric oxide (125 mg/kg; intraperitoneal) daily 30 min before sesamol (8 mg/kg; oral gavage) for 21 days. In another set of experiments, we investigated the synergistic effect of sesamol (4 mg/kg; oral gavage) with L-NAME, a non specific nitric oxide synthase inhibitor (10 mg/kg; intraperitoneal). L-NAME was administered daily 30 min before sesamol (4 mg/kg; oral gavage) for 21 days.

2.5. Behavioral tests

2.5.1. Morris water maze test

Animals were tested in a spatial version of Morris water maze test (Morris et al., 1982; Tuzcu and Baydas, 2006). The apparatus consisted of a circular water tank (180 cm in diameter and 60 cm high). A platform (12.5 cm in diameter and 38 cm high) invisible to the rats, was set 2 cm below the water level inside the tank with water maintained at 28.5±2 °C at a height of 40 cm. The tank was located in a large room where there were several brightly colored cues external to the maze; these were visible from the pool and could be used by the rats for spatial orientation. The position of the cues remained unchanged throughout the study. The water maze task was carried out for five consecutive days from 15th to 19th day. The rats received four consecutive daily training trials in the following 5 days, with each trial having a ceiling time of 90 s and a trial interval of approximately 30 s. For each trail, each rat was put into the water at one of four starting positions, the sequence of which being selected randomly. During test trials, rats were placed into the tank at the same starting point, with their heads facing the wall. The rat had to swim until it climbed onto the platform submerged underneath the water. After climbing onto the platform, the animal remained there for 20 s before the commencement of the next trial. The escape platform was kept in the same position relative to the distal cues. If the rat failed to reach the escape platform within the maximally allowed time of 90 s, it was guided with the help of a rod and allowed to remain on the platform