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Naringin ameliorates memory deficits in experimental paradigm of Alzheimer's disease by attenuating mitochondrial dysfunction

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ABSTRACT

Rationale: Mitochondrial dysfunction has been well documented in age related disorders like Alzheimer's disease. Alterations in mitochondrial membrane potential lead to neuronal death by excessive generation of free radicals, inflammatory cytokines, and excitotoxins. Intracerebroventricular (ICV) streptozotocin (STZ) induced-cognitive impairment has been widely used as an experimental model of Alzheimer's disease. Naringin is a potent antioxidant, which can cross the blood brain barrier protecting brain tissue and modulating brain chemistry.
Objectives: The present study was designed to evaluate the effect of naringin, in ICV STZ-induced mitochondrial dysfunction and memory loss in rats.
Methods: Streptozotocin (3 mg/kg, ICV) was injected bilaterally in two divided doses on first and third day followed by treatment with different doses of naringin (50, 100 and 200 mg/kg; p.o.) for twenty one days. Behavioral alterations were monitored using Morris water maze paradigm and elevated plus maze test. Animals were sacrificed to evaluate various biochemical and mitochondrial parameters in brain. Rivastigmine was used as a standard drug.
Results: ICV-STZ administration produced significant cognitive deficits as assessed by both Morris water maze and elevated plus maze task which is accompanied by significantly enhanced oxidative-nitrosative stress, altered acetylcholinesterase and mitochondrial enzyme activities in cerebral cortex and hippocampus of rats brain along with significantly increased brain TNF-α and IL-1β levels. Chronic treatment with naringin dose dependently restored cognitive deficits in ICV-STZ rats along with mitigation of mitochondrial dysfunction mediated oxido-nitrosative stress and cytokine release.
Conclusions: Our findings demonstrate that naringin ameliorates mitochondrial dysfunction mediated oxido-nitrosative stress and inflammatory surge in ICV-STZ treated rats.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by progressive cognitive decline, widespread loss of neurons/neuronal synapses in the cerebral cortex and hippocampus. Mitochondria are uniquely poised to play a pivotal role in neuronal cell survival/death because they are regulators of both energy metabolism and cell death pathways (Moreira et al., 2010)

Recent evidence reveals that mitochondrial dysfunction mediated oxidative stress plays an important role in the early pathology of AD (Moreira et al., 2010). Being the major source of Reactive oxygen species (ROS), mitochondria are subjected to direct attack by large amounts of ROS in the cell and might be therefore particularly susceptible to oxidative damage (Eckert et al., 2003). As a consequence, damaged mitochondria progressively become less efficient, losing their functional integrity and release more reactive oxygen molecules (Reddy, 2007). Other consequences of mitochondrial dysfunction include reduction in mitochondrial ATP production, increased mitochondrial DNA mutations, increase in abnormal mitochondrial criste structures and impaired intracellular calcium levels (Reddy and Beal, 2005). Increased ROS generation with compromised mitochondrial function ultimately affects neurons and accelerates neurodegenerative process (Zeevalk et al., 2005).

Intracerebroventricular-streptozotocin (ICV-STZ) injection in subdiabetogenic dose in rats has been described as an appropriate animal model for sporadic type Alzheimer disease and characterized by progressive cognitive dysfunction due to reduced energy metabolism/oxidative stress by inhibiting the synthesis of adenosine triphosphate (ATP) and acetyl-CoA (Sharma and Gupta, 2001a,b). This ultimately results in cholinergic deficiency supported by reduced cholineacetyltransferase (ChAT) activity in hippocampus and an increased cholinesterase (ChE) activity in the brain of ICV-STZ rats (Blokland and Jolles, 1993; Sharma and Gupta, 2001a,b; Sonkusare et al., 2005).

Bioflavonoids are a ubiquitous group of polyphenolic substances present in most plants and are frequently consumed in human diet (Nijveldt et al., 2001). The widespread distribution of flavonoids, coupled with relatively low toxicity compared to other active plant

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compounds (for instance alkaloids) makes them potential candidates to be developed as therapeutic entities. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers" (Nakagami et al., 1995). It has been reported that flavonoids exert beneficial effects in experimental models of memory impairment due to their strong antioxidant and anti-inflammatory potential (Baluchnejadmojarad and Roghani, 2006; Tota et al., 2010). Naringin (4',5,7-trihydroxyflavanone 7-rhamnoglucoside) is a well-known flavanone glycoside of grape fruits, e.g., *Citrus paradise, Citrus sinensis, Citrus unshiu,* and *Artemisia selengensis* (Kumar et al., 2010), roots of *Cudrania cochinchinensis* and fruits of Pon cirus. Kandhare et al. (2012) demonstrated a neuroprotective effect of naringin by modulation of endogenous biomarkers and down regulation of free radical, cytokine including tumor necrosis factor-α (TNF-α), in streptozotocin induced painful diabetic neuropathy. Naringin or its metabolite has been reported to possess diverse biological and pharmacological properties including anticarcinogenic (So et al., 1996), lipid-lowering (Jeon et al., 2004), superoxide scavenging (Rajadurai and Prince, 2009), anti-apoptotic (Kim et al., 2009), anti-atherogenic (Choe et al., 2001), metal chelating (Jagetia et al., 2003) and antioxidant activities (Jagetia and Reddy, 2005). Some growing evidence has indicated that naringin displays anti-inflammatory effects both in *in-vitro* and *in-vivo* systems (Kanno et al., 2006).

Orally administered naringin is metabolized to naringenin (4', 5, 7-trihydroxyflavanone) (Fuhr and Kummert, 1995) which crosses the blood brain barrier (Zbarsky et al., 2005). With this background, the present study was designed to explore the possible role of naringin against ICV-STZ induced mitochondrial dysfunction mediated memory deficits, oxido-nitrosative stress and inflammatory surge in rats and acetylcholinesterase inhibitor, rivastigmine used as standard.

2. Material and Methods

2.1. Animals

Adult male Wistar rats (200–230 g, 3 months old) bred in Central Animal House facility of Panjab University were used. The animals were housed under standard laboratory conditions, maintained on a 12:12 h light:dark cycle and had free access to food (Ashirwad Industries, Mohali, India) and water. Animals were acclimatized to laboratory conditions before all the behavioral tests. All experiments were carried out between 0900 and 1700 h. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC/282/UIPS/15 dated 30/8/12) of Panjab University and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation.

2.2. Drugs & treatment

Naringin, Rivastigmine and Streptozotocin were purchased from Sigma Aldrich, St. Louis, MO, USA. Tumor necrosis factor-α (TNF- α), Interleukin-1 beta (IL-1β) ELISA kits were purchased from R&D Systems, Minneapolis, MN, USA. Naringin was dissolved in double distilled water while streptozotocin was dissolved in artificial cerebrospinal fluid (aCSF) (2.9 mM KCl, 147 mM NaCl, 1.7 mM $CaCl_2$, 1.6 mM $MgCl_2$, and 2.2 mM D-glucose). All drug solutions were freshly prepared immediately prior to injection. Naringin (50 mg/kg, 100 mg/kg and 200 mg/kg (Aggarwal et al., 2010) and Rivastigmine [2 mg/kg; (Singh and Chopra, 2014)] were administered by oral gavage daily for 21 days. All other chemicals used were of analytical grade.

2.3. Surgical procedures: ICV injection of STZ

ICV streptozotocin was injected intracerebroventricularly according to the procedure of Sonkusare et al. (2005). Rats were anesthetized with thiopentone (Neon Laboratories, India) at a dose of 45 mg/kg, i.p. 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 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, ICV) was dissolved in aCSF and injected bilaterally in two divided doses on first and third day 1.5 mg/kg each day. The concentration of STZ in aCSF was adjusted so as to deliver 3 μl of the solution. On first day just after STZ administration; a cannula was implanted at the site of injection and on 3rd day the cannula was removed, STZ was administered and wound was sutured followed by daily application of antiseptic powder (Neosporin®). One sham group was also included in the study to nullify the effect of surgery, if any. Sham animals received ICV injection of the same volume of aCSF on the first and third day. 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 assigned to seven different groups containing 5–8 animals in each group viz Group 1: control animals received distilled water; Group 2: sham-operated animals received ACSF (ICV, 3 μl) on day 1 and day 3; Groups 3: animals received ICV-STZ 1.5 mg/kg on day 1 and day 3 each; Groups 4, 5 and 6: ICV-STZ rats being administered naringin (50 mg/kg, 100 mg/kg and 200 mg/kg; oral gavage) respectively for 21 days; Groups 7: ICV-STZ rats being administered rivastigmine (2 mg/kg oral gavage) as standard for 21 days. Memory impairment was assessed by Morris Water Maze on days 15th to 19th and elevated plus maze on days 20th and 21st. Locomotor activity was measured on day 22. After behavioral experiments, rats were anesthetized with thiopentone sodium (40 mg/kg; i.p.) and blood was collected through tail vein followed by decapitation of animals by cervical dislocation. Brains were quickly removed, cleaned with chilled saline and stored at -80 °C till further analysis (Fig. 1).

2.5. Behavioral tests

2.5.1. Morris water maze (computer tracking using EthoVision software)

Animals were tested in a spatial version of Morris water maze test for assessment of memory (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 30s. For each trial, 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 20s 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 90s, it was guided with the help of a rod and allowed to remain on the platform for 20s. The time to reach the platform (escape latency in seconds) and total distance travelled to reach the hidden platform (path length in cm) was measured by using computer tracking system with EthoVision software (Noldus Information Technology, Wageningen, Netherlands).

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