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Lycopene protects against memory impairment and mito-oxidative damage induced by colchicine in rats: An evidence of nitric oxide signaling

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ABSTRACT

Oxidative–nitrosative stress and mitochondrial dysfunction plays an important role in the onset of various neurodegenerative diseases. Lycopene, a carotenoid antioxidant, has received considerable scientific interest in recent years. Present study was designed to evaluate the possible nitric oxide mechanism in protective effects of lycopene against the colchicine induced cognitive impairment and mito-oxidative damage in rats. Wistar rats were received i.c.v. colchicine (15 μg/5 μl). Lycopene (2.5 and 5 mg/kg), NO modulators e.g. L-Arginine (50 mg/kg) L-NAME (5 mg/kg) administered for 21 days. Behavioural alterations were assessed in between study period. Animals were killed immediately following the last behavioral session, and mitochondrial enzymes, oxidative parameters, inflammatory mediators (TNF-α, IL-6) and caspase-3 activity were measured. I.C.V. administration of colchicine impaired memory performance in Morris water maze, oxidative defense and mitochondrial complex enzymes activities as compared to sham group. A significant increase of TNF-α, IL-6 and caspase-3 activity in hippocampus and cortex was also noted. Chronic treatment lycopene significantly improved memory retention and attenuated mito-oxidative damage parameters, inflammatory markers and apoptosis in colchicine treated rats. Further, L-arginine pretreatment with sub effective dose of lycopene significantly reversed the protective effect of lycopene. However, L-NAME pretreatment with sub effective dose of lycopene significantly potentiated the protective effect of lycopene which was significant as compared to their effect per se. These results suggest that lycopene exhibit a neuroprotective effect by accelerating brain anti-oxidant defense mechanisms and down regulating nitric oxide pathways. Thus, lycopene may be used as therapeutic agent in preventing complications in memory dysfunction.

1. Introduction

Colchicine is potent microtubule-disrupting agent and having strong cytotoxic properties, such as cell mitosis inhibition and induction of microcytoskeleton depolymerization. The clinical manifestations of colchicine toxicity are characterized by multi-organ failure, myocardial toxicity, bone marrow suppression, renal failure, hypocalcemia, and rhabdomyolysis (Bouquie et al., 2011; Montiel et al., 2010). Among all, the colchicine induced neurotoxicity is one of the main pathological conditions of many neurodegenerative disorders. However, it is revealed that colchicine is selectively toxic to certain neuronal populations in the CNS, particularly granule cells of the dentate gyrus in hippocampus (Goldschmidt and Steward, 1980). It is widely accepted that oxidative damage is the main culprit implicated in pathology of the various neurodegenerative disorders (Verri et al., 2012). However, it remains to be clarified just how the oxidative stress is activated or initiated. One characteristic common for initiating oxidative stress to all neurodegenerative diseases is free radical production at the mitochondrial level (Zhu et al., 2006). Furthermore, apart of oxidative stress and mitochondria orchestrate, the apoptotic pathway which is activated by cytochrome *c*, is the main cause of neuronal cell death (Krantic et al., 2005). It is well known that colchicine triggers apoptosis in several neuronal in vitro models such as organotypic hippocampal slice cultures (Kristensen et al., 2003).

Recent findings indicate a pivotal role for NO and pro-oxidants in neuronal functional impairment and the related structural damage. This is mainly caused by oxidative/nitrosative effects due to the extensive release of NO. However, it can form peroxynitrite through a diffusion-limited reaction with superoxide, creating a more potent and specific oxidant (Beckman and Koppenol, 1996; Crow and Beckman, 1996). It has shown that the expression of different NOS activity has been increased in both central and peripheral neurons

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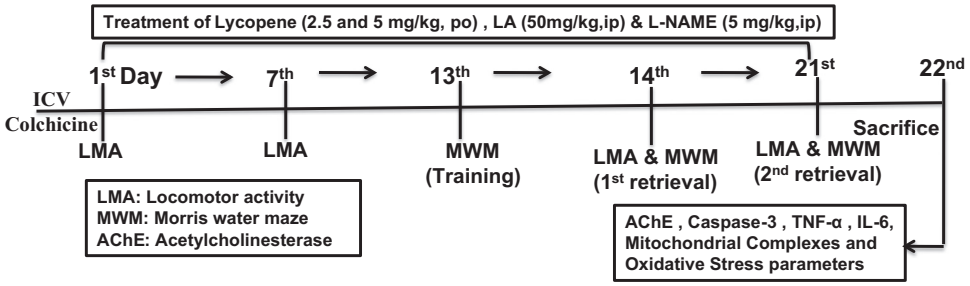

Scheme 1. Protocol design.

after axonal injury (Wu et al., 1994; Vizzard et al., 1995; Wu, 1996). It has been reported that colchicine causes the increase in expression of NOS, in central neurons and stimulates the production of NO (Bonfoco et al., 1995), Further, Dufourny et al. (2000) reported that intracerebroventricular injection of colchicine increased the number of NOS-containing cells in the hypothalamic nuclei of guinea pigs.

Since past decade, the scientific community has shown huge interest in the efficacy of herbal remedies. So the concept of complementary or alternative medicine is becoming much more widely accepted. The antioxidant capabilities of lycopene have been documented previously in different disease animal model. In addition to, lycopene has been reported to have potent neuroprotective (Hsiao et al., 2004), antiproliferative, anticancer (Gunasekera et al., 2007), anti-inflammatory (Kuhad et al., 2008) and cognition enhancing (Akbaraly et al., 2007). However, its property has not yet been evaluated against colchicine induced neurotoxicity. Since oxidative damage is the main target, we wanted to investigate the downstream signaling pathways of lycopene mediated reversal of colchicine induced cognitive dysfunction.

2. Materials and methods

2.1. Animals

Male Wistar rats (weighing 180–200 g) (Central Animal House, Panjab University, Chandigarh) were used. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. Following surgery, animals were kept under standard conditions of a 12 h light/dark cycle with food and water ad libitum in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 17.00 h. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

2.2. Surgery and intracerebroventricular administration of colchicine

Surgery was performed as per the previously described protocol (Prakash and Kumar, 2009) All animals were anesthetized with thiopental sodium (45 mg/kg, i.p.) and positioned in a stereotaxic apparatus. The head was positioned in a frame and a midline sagittal incision was made in the scalp and the skull was adjusted to place bregma and lambda on the same horizontal plane. Two holes were drilled in the skull for the placement of the injection cannula on both sides over the bilateral cerebral ventricle using following coordinates as described by Paxinos and Watson: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm beneath the cortical surface of brain. The scalp was then closed with a suture and dental cement. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent sepsis after post surgery. Animals were given bilateral i.c.v. injection of colchicine (15 μg/5 μl) with help of Hamilton micro syringe through cannula at rate of 1 μl/min. This colchicine solution was made freshly by the help of saline just before injection. To promote the diffusion the micro syringe was left in place for a period of 5 min following injection. Special care of the animals was taken during the post-operative period.

2.3. Drugs and treatment schedule

Colchicine, nitro-L-arginine methyl ester (L-NAME), L-arginine (L-Arg) and lycopene were purchased from Sigma Chemicals Co., St. Louis, Mo, USA. Colchicine was prepared in saline such that a 15 μg dose was delivered in a 5 μl injection volume for i.c.v. administration. For oral administration, lycopene was suspended in 0.5% sodium carboxy methyl cellulose (CMC) and administered in a dose of 0.5 ml/100 g body weight. L-NAME (5 mg/kg) and L-Arg (50 mg/kg) were dissolved in normal saline and administered intraperitoneally. Animals were divided in several groups (mentioned below), consist of eight animals in each. Study was performed in multiple phases as per shown in experimental protocol (Scheme 1). The doses of colchicine and lycopene were selected based on the previous studies in our laboratory.

Group 1—Sham-operated (received vehicle for lycopene)
Group 2—Lycopene (5 mg/kg) per se
Group 3—Colchicine treated group (15 μg/5 μl i.c.v.)+vehicle for lycopene
Groups 4 and 5—Lycopene (2.5 and 5 mg/kg, p.o.)+Colchicine
Group 6—L-Arginine (50 mg/kg i.p.)+Lycopene (2.5 mg/kg, p.o.)+Colchicine
Group 7—L-NAME (5 mg/kg i.p.)+Lycopene (2.5 mg/kg, p.o.)+Colchicine.

2.4. Behavioral assessment

2.4.1. Spatial navigation task

The acquisition and retention of a spatial navigation task was evaluated by using Morris water maze (Prakash and Kumar, 2009). Animals were trained to swim to a visible platform in a circular pool (180 cm in diameter and 60 cm in height) located in a test room. In principle, rats can escape from swimming by climbing onto the platform and over time the rats apparently learn the spatial location of the platform from any starting position at the circumference of the pool. The pool was filled with water (28 ± 2 °C) to a height of 40 cm. a movable circular platform (9 cm diameter), mounted on a column was placed in a pool 2 cm above the water level during the acquisition phase. A similar platform was placed in the pool 2 cm below the water level for the maze retention phase. During both the phases, the platform was placed in the centre of one of the quadrants. Four equally spaced locations around the edge of the pool (N, S, E, and W) were used as starting points and this divided the pool into four equal quadrants.