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Sesamin attenuates behavioral, biochemical and histological alterations induced by reversible middle cerebral artery occlusion in the rats

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ABSTRACT

Restoration of blood flow to an ischemic brain region is associated with generation of reactive oxygen species (ROS) with consequent reperfusion injury. ROS cause lipid peroxidation, protein oxidation, and DNA damage, all of which are deleterious to cells. So diminishing the production of free radicals and scavenging them may be a successful therapeutic strategy for the protection of brain tissue in cerebral stroke. The present study investigated the neuroprotective effect of sesamin (Sn) to reduce brain injury after middle cerebral artery occlusion (MCAO). The middle cerebral artery (MCA) of adult male Wistar rat was occluded for 2 h and reperfused for 22 h. Sesamin is the most abundant lignan in sesame seed oil is a potent antioxidant. Sesamin (30 mg/kg) was given orally twice, 30 min before the onset of ischemia and 12 h after reperfusion. The initial investigations revealed that sesamin reduced the neurological deficits in terms of behavior and reduced the level of thiobarbituric acid reactive species (TBARS), and protein carbonyl (PC) in the different areas of the brain when compared with the MCAO group. A significantly depleted level of glutathione and its dependent enzymes (glutathione peroxidase [GPx] and glutathione reductase [GR]) in MCAO group were protected significantly in MCAO group treated with sesamin. The present study suggests that sesamin may be able to attenuate the ischemic cell death and plays a crucial role as a neuroprotectant in regulating levels of reactive oxygen species in the rat brain. Thus, sesamin may be a potential compound in stroke therapy.

1. Introduction

Stroke is the third leading cause of death and the leading cause of severe long-term disability in developed countries [1]. Ischemic stroke is characterized by the sudden loss of blood circulation to an area of the brain, resulting in a corresponding loss of neurologic function. The complex pathobiological mechanisms of this medical problem include excitotoxicity, inflammation, apoptosis, oxidative damage and ionic imbalances [2–4]. These changes are associated with mitochondrial dysfunction and rapid decreases in ATP, resulting free radical generation and lipid peroxidation.

Brain tissue has potential sources of phospholipid and high amounts of polyunsaturated fatty acids (PUFA) and large oxidative capacity, but its ability to combat oxidative stress is limited [5]. Increasing evidence suggests that oxidative stress is a prominent and early feature in the pathogenesis of cerebral ischemia [6–8]. Thus, it can be speculated that pharmacological agents with antioxidant property may boost the system to stay normal against the oxidative damage. Earlier our research group has investigated and reported the preventive effect of certain antioxidants against experimental models of stroke [4,9–11].

Furthermore, ROS also mediate a mitochondrial signalling pathway that may lead to apoptosis following cerebral ischemia [12–14]. Caspases play a key role in the execution phase of apoptosis by cleaving specific proteins resulting in an irreversible commitment to cell death. Activation of caspase-3-like proteases is likely to be relevant in neuronal apoptosis and in ischemic brain injury [15].

Experimental models of cerebral ischemia have been developed to improve our understanding of the deleterious mechanisms

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involved in brain ischemic damage, and to study the potential efficiency of therapeutic strategies. The focal cerebral ischemia model with transient occlusion is being generally accepted as the model that most closely duplicates stroke in human patients.

It has been a major challenge to develop effective therapeutics for stroke. Many antioxidants are reported to reduce reactive oxygen species-mediated reactions and rescue neurons from ischemia-reperfusion-induced neural loss in animal models of cerebral ischemia [4,11,16]. Sesamin is the most abundant lignan in sesame seed oil [17]. Sesamin is assumed to play important roles in plant defence and have been frequently used as potent antioxidants [18]. Sesamin enhances hepatic detoxification, reduces the chemical-induced tumor, and protects against oxidative stress [19–22]. Neuroprotection of sesamin was attributed to their antioxidant and anti-inflammatory properties [23–26]. Recently Ahmad et al. in our research group have reported the neuroprotective effect of sesame oil for the treatment of cerebral ischemia [11]. Thus, sesamin which is the important lignan of sesame oil and its above properties has stimulated us to study its neuroprotective role in ischemia-reperfusion injury. Accordingly, we designed this study to investigate the effects of sesamin on the post ischemic deterioration in stroke model of rat.

2. Materials and methods

2.1. Chemicals and reagents

Oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), 2,3,5-triphenyltetrazolium chloride (TTC), ethylene diamine tetra-acetic acid (EDTA), 2,4-dinitrophenylhydrazine (DNPH), (−)epinephrine, paraformaldehyde, glycine, and diaminobenzidine (DAB), were purchased from Sigma–Aldrich chemicals Pvt. Ltd., India. Monoclonal p53 antibody was purchased from Bio Vision, and anti-mouse IgG was purchased from Jackson Immuno Research Laboratories Inc., West Groove, PA. ATP kit was procured from Sigma–Aldrich Chemicals Pvt. Ltd., USA. Sesamin complex was a generous gift from M/s Sami Lab Ltd., Bangalore, India.

2.2. Animals and treatments

Male Wistar rats weighing 250–270 g (approx. 16 weeks old) were used. They were kept in the Central Animal House of Hamdard University in colony cages at an ambient temperature of 25 ±2 °C and relative humidity 45–55% with 12 h light/dark cycles. They had free access to standard rodent pellet diet and water *ad libitum*. The food was withdrawn 12 h before the surgical procedure. Experiments were conducted in accordance with the Animal Ethics Committee of the University.

2.3. Experimental protocol

To investigate the neuroprotective effects of sesamin in experimental model of cerebral ischemia, we used the rat MCAO model [27]. Animals were divided into four groups each having eight animals. The first group served as sham and vehicle was given orally, second was middle cerebral artery occluded (MCAO), i.e., ischemia was induced for 2 h followed by reperfusion for 22 h, third was treated with sesamin (30 mg/kg in olive oil, orally) 30 min before the onset of ischemia and 12 h after reperfusion (i.e., Sn + L group) and fourth was treated for twice with drug alone, i.e., sesamin group (30 mg/kg, orally). After the completion of the reperfusion period, the animals were assessed for neurobehavioral activity and then sacrificed. The brains were taken out to dissect hippocampus and frontal cortex for biochemical estimations.

2.4. Surgical procedure

The right middle cerebral artery occlusion was performed using an intraluminal filament model by the method of Longa et al. [27] as described by us [9]. In brief, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), our silicone rubber (4 0-3033REPK10, DOCEOL, USA) coated monofilament has a smooth, soft, and flexible tip (which reduces the risk of vessel injury and intracranial bleeding) was introduced into the external carotid artery (ECA) and advanced into the middle cerebral artery via the internal carotid artery (ICA) (17–20 mm) until a slight resistance was felt. Such resistance indicated that the filament had passed beyond the proximal segment of the anterior cerebral artery (ACA). At this point, the intraluminal suture blocks the origin of MCA and occluded all sources of blood flow from ICA, anterior cerebral artery and the posterior cerebral artery. Two hours after the induction of ischemia, the filament was slowly withdrawn and the animals were then returned to their cages. In the groups of sham-operated rats all surgical procedures except the MCAO were performed. The animals were then returned to their cages and given free access to food and water.

2.5. Evaluation of ischemic damage

2.5.1. Neurological deficits

After 22 h of reperfusion, the neurological status of the animals was evaluated using two different methods: method A was used as previously described by Bederson et al. [28]. Accordingly, four categories of neurological findings were scored: 0 = no observed neurological deficit; 1 = contralateral forelimb flexion with wrist flexion and shoulder adduction; 2 = reduced resistance to lateral push; 3 = circling movements towards the paretic side. In method B, spontaneous motor activity (SMA) was evaluated for 5 min by placing the animals in their normal environment (cage). Neurological scoring was given as: 0 = rats moved around in the cage and explored the environment; 1 = rats moved in the cage but did not approach to all the sides and hesitated to move; 2 = rats barely moved in the cage and showed postural abnormalities (curved towards the paretic side); 3 = rats unable to move at all with their posture curved towards the paretic side.

2.5.2. Rota rod (muscular coordination)

Omni Rotor (Omnitech Electronics Inc., Columbus, OH, USA) was used to evaluate the muscular coordination after 24 h [29]. It consisted of a rotating rod, 75 mm diameter, which was divided into four parts by compartmentalization to permit the testing of four rats at a time. The apparatus automatically records the time in 0.1 s when the rats fall of the rotating shaft. The speed was set at 10 rpm and cut-off time was 180 s, and the drug-naive animals were trained on the rod, so that they could stay on it at least for the cut-off time.

2.6. Biochemical studies

2.6.1. Tissue preparation

After behavioral study, the animals were sacrificed and their brains were taken out to dissect hippocampus and frontal cortex to give 5% (w/v) homogenate (10 mM phosphate buffer, pH 7.0 having 10 μl/ml protease inhibitors: 5 mM leupeptin, 1.5 mM aprotinin, 2 mM phenylethylsulfonylfluoride (PMSF), 3 mM peptastatin A, 10 mM EDTA, 0.1 mM EGTA, 1 mM benzamidine and 0.04% butylated hydroxytoluene) and were centrifuged at 1000 × *g* for 5 min at 4 °C to separate debries. This supernatant was used for the assay of TBARS. The rest of the supernatant was centrifuged at 10,500 × *g*