NeuroToxicology 45 (2014) 100–110

Contents lists available at ScienceDirect

NeuroToxicology

Sesamin attenuates neurotoxicity in mouse model of ischemic brain stroke

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ARTICLE INFO

Article history:
Received 22 July 2014
Accepted 2 October 2014
Available online 12 October 2014

Keywords:
Sesamin
Stroke
Antioxidants
Inflammation
MAPKinase ameliorate

ABSTRACT

Stroke is a severe neurological disorder characterized by the abrupt loss of blood circulation into the brain resulting into wide ranging brain and behavior abnormalities. The present study was designed to evaluate molecular mechanism by which sesamin (SES) induces neuroprotection in mouse model of ischemic stroke. The results of this study demonstrate that SES treatment (30 mg/kg bwt) significantly reduced infarction volume, lipid per-oxidation, cleaved-caspase-3 activation, and increased GSH activity following MCAO in adult male mouse. SES treatment also diminished iNOS and COX-2 protein expression, and significantly restored SOD activity and protein expression level in the ischemic cortex of the MCAO animals. Furthermore, SES treatment also significantly reduced inflammatory and oxidative stress markers including Iba1, Nox-2, Cox-2, peroxynitrite compared to placebo MCAO animals. Superoxide radical production, as studied by DHE staining method, was also significantly reduced in the ischemic cortex of SES treated compared to placebo MCAO animals. Likewise, downstream effects of superoxide free radicals i.e. MAPK/ERK and P38 activation was also significantly attenuated in SES treated compared to placebo MCAO animals. In conclusion, these results suggest that SES induces significant neuroprotection, by ameliorating many signaling pathways activated/deactivated following cerebral ischemia in adult mouse.

1. Introduction

Ischemic stroke is the second leading cause of death worldwide with a high incidence of permanent disability in surviving individuals (Liu et al., 2014). Ischemic stroke is caused by reduction or complete blockade of cerebral blood flow owing to thrombi or embolic which reduces oxygen and energy supply to the critical tissues of the brain, resulting in rapid cell death and a corresponding loss of neurologic function (Pradeep et al., 2012). Cerebral ischemia/reperfusion insult produces damage of brain tissues via complex rapid pathophysiological mechanisms including glutamate excitotoxicity, inflammation as a result of the blood–brain barrier disruption, oxidative damage, loss of ionic homeostasis, phospholipase activation (Nito et al., 2008), neuronal depolarization (Sun et al., 2013) and finally apoptosis (Diaz-Ruiz et al., 2014).

Because of the devastating consequences and disabilities associated with stroke survivors that cause a significant social and economic burden on societies worldwide, ischemic stroke is

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http://dx.doi.org/10.1016/j.neuro.2014.10.002
0161-813X/

nowadays a major public health concern (Yuen et al., 2011). Despite current advances in thrombolytic therapy for acute ischemic stroke, its clinical application is limited because of possible association with symptomatic intracranial hemorrhage and poor functional outcomes (Whiteley et al., 2014), therefore there is a great need for designing new therapeutic strategies, which can provide neuroprotection in stroke.

Recently, attention has been focused on natural products as natural sources to develop safe and potent agents for enhancing the survival of neurons and preventing ischemia-induced neuronal death (Lim et al., 2013). Sesamin (SES) is one of the most abundant ligand in sesame seeds (Phitak et al., 2012). It has been shown to exhibit multiple biological functions, such as inhibition of inflammation (Lin et al., 2014), carcinogenesis (Deng et al., 2013) and oxidative stress (Hsieh et al., 2011). In addition, sesamin has been reported to attenuate hypertension (Matsumura et al., 1995), serum and hepatic cholesterol (Hirata et al., 1996), serum triglycerides (Fukuda et al., 1998), cerebral thrombogenesis (Noguchi et al., 2004), and neuroinflammation (Bournival et al., 2012b). Further, sesamin also exhibited neuroprotective effects in various models of neuronal cell injuries caused by ischemia or oxidative stress (Hou et al., 2003, 2004; Zhang et al., 2012a). Earlier we demonstrated neuroprotective effect of sesamin in ischemia-reperfusion injury induced in rats by reversible middle cerebral artery occlusion (Khan et al., 2010) however a detailed mechanism has not been presented. In the present study, we have elucidated detail mechanisms underlying the neuroprotective effects of sesamin, which will help in understanding potential pathogenic pathways and therapeutic mechanisms of SES in ischemia-induced brain injury.

2. Methods

2.1. Animals and experimental design

Male mice in C57BL/6 (Jackson Laboratory, Bar Harbor, ME) background were used in all experiments. The mice were kept under standard environmental and nutritional conditions throughout the investigation. Animals had free access to standard rodent pellet diet and water *ad libitum*. 12 h before the surgical procedure food was withdrawn. This study was performed in accordance with the Public Health Service Guide for the Care and Use of Laboratory Animals (Department of Health, Education, and Welfare, National Institutes of Health), Georgia Regents University, and King Abdulaziz university guidelines. Sesamin dose was already established in our earlier study (Khan et al., 2010). Mice were randomly distributed into 3 major groups (15 mice each group) as follows: Group I: Sham operated mice and vehicle was given as i.p., Group II: Ischemic mice were middle cerebral artery occluded (MCAO), i.e., ischemia was induced for 2 h followed by reperfusion for 22 h, Group III: Ischemic mice treated with sesamin (SES, 30 mg/kg bwt in 0.1% DMSO, i.p.) 30 min before the onset of ischemia and 12 h after reperfusion (MCAO+ SES).

2.2. Surgical procedure and collection of sample

Middle cerebral artery occlusion, (MCAO) was performed according to the method described previously by (Ahmad et al., 2006). In brief, Mice were anesthetized with chloral hydrate (400 mg/kg bwt, i.p.); silicone rubber (4 0-3033REPK10, DOCEOL, USA) coated monofilament has a smooth, soft, and flexible tip 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.

After the completion of the reperfusion period, the animals were anesthetized with chloral hydrate (400 mg/kg bwt, i.p.) and perfused transcardially through ascending aorta, with in cold phosphate buffer saline (PBS; 0.1 M, pH 7.2). The brains were collected for biochemical estimations. For histological study we perfused the mice with 4% paraformaldehyde in 1 X PBS and brains were collected for the sectioning (Ahmad et al., 2012).

2.3. Preparation of brain homogenate

The brains were homogenized according to previously described method (Ahmad et al., 2006) to prepare 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* for 15 min at 4 °C to separate post mitochondrial supernatant (PMS), which was used for the estimation of GSH and superoxide dismutase activity.

2.4. Estimation of thiobarbituric acid reactive substances (TBARS)

TBARS activity assay was done according to method described previously by us with slight modification (Ahmad et al., 2012). The homogenate 0.25 mL was incubated at 37 ± 1 °C in a metabolic shaker (120 strokes/min) for 1 h. Similarly, 0.25 mL of the same homogenate was pipetted in a test tube and incubated at 0 °C. After 1 h of incubation, 0.5 mL of 5% chilled trichloroacetic acid (TCA) was added followed by 0.25 mL of 0.67% thiobarbituric acid (TBA) in each test tube and proper mixing was done after each addition. The mixture was centrifuged at 3000 × *g* for 10 min and supernatant was transferred to another test tube and placed in boiling water bath for 10 min. The test tubes were put to cool and the absorbance of the color was read at 535 nm. The rate was expressed as nmol TBARS formed/h/mg protein using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.5. Reduced glutathione (GSH) content measurement

GSH content was determined according to the previously described method (Jollow et al., 1974) with slight modification (Ahmad et al., 2006). PMS was mixed with 4.0% sulfosalisylic acid in a 1:1 ratio (v/v). The samples were incubated at 4 °C for1 h, and later centrifuged at 1200 × *g* for 15 min at 4 °C. The assay mixture contained 0.1 mL of supernatant, 1.0 mM 5-5′-dithio-bis-2-nitrobenzoic acid (DTNB) and 0.1 M PB (pH 7.4) in a total volume of 1.0 mL. The yellow color developed was read immediately at 412 nm using a spectrophotometer (UV-1601, Shimadzu, Japan). The GSH content was calculated as nmol of DTNB conjugate formed/mg protein using a molar extinction coefficient of $13.6 \times 10^3\ M^{-1}\ cm^{-1}$.

2.6. Superoxide dismutase (SOD) activity assay

SOD activity was measured according to the method described previously (Stevens et al., 2000) by monitoring the auto oxidation of (−)-epinephrine at pH 10.4 for 3 min at 480 nm. The reaction mixture contained glycine buffer (50 mM, pH, 10.4) and 0.2 mL of