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S-allyl cysteine mitigates oxidative damage and improves neurologic deficit in a rat model of focal cerebral ischemia☆

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Abstract

Oxidative stress and inflammatory damage play an important role in cerebral ischemic pathogenesis and may represent a target for treatment. The present study examined the hypothesis that S-allyl cysteine (SAC), organosulfur compounds found in garlic extract, would reduce oxidative stress–associated brain injury after middle cerebral artery occlusion (MCAO). To test this hypothesis, male Wistar rats were subjected to MCAO for 2 hours and 22-hour reperfusion. S-allyl cysteine was administered (100 mg/kg, b.wt.) intraperitoneally 30 minutes before the onset of ischemia and after the ischemia at the interval of 0, 6, and 12 hours. After 24 hours of reperfusion, rats were tested for neurobehavioral activities and were killed for the infarct volume, estimation of lipid peroxidation, glutathione content, and activity of antioxidant enzymes (glutathione peroxidase, glutathione reductase, catalase, and superoxide dismutase). S-allyl cysteine treatment significantly reduced ischemic lesion volume, improved neurologic deficits, combated oxidative loads, and suppressed neuronal loss. Behavioral and biochemical alterations observed after MCAO were further associated with an increase in glial fibrillary acidic protein and inducible nitric oxide expression and were markedly inhibited by the treatment with SAC. The results suggest that SAC exhibits exuberant neuroprotective potential in rat ischemia/reperfusion model. Thus, this finding of SAC-induced adaptation to ischemic stress and inflammation could suggest a novel avenue for clinical intervention during ischemia and reperfusion.

Keywords: S-allyl cysteine; Middle cerebral artery occlusion; Neurobehavior; Oxidative damage; Inflammation

Abbreviations: DAB, diaminobenzidine; EDTA, ethylenediamine tetra acetic acid; EGTA, ethylene glycol tetraacetic acid; GFAP, glial fibrillary acidic protein; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; CAT, catalase; iNOS, inducible nitric oxide synthase; LPO, lipid peroxidation; MCAO, middle cerebral artery occlusion; NADPH, nicotinamide adenine dinucleotide phosphate; PB, phosphate buffer; PBS, phosphate buffer saline; PMS, postmitochondrial supernatant; ROS, reactive oxygen species; SAC, S-allyl cysteine; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TTC, 2,3,5-triphenyltetrazolium chloride.

☆ Conflict of interest. Authors have no conflict of interest.

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1. Introduction

Stroke is a global public-health problem, which causes disability and, in severe cases, death as well. Ischemic stroke is caused by obstruction of blood flow to the brain, resulting in energy failure that initiates a complex series of metabolic events, ultimately causing neuronal cell death. The ensuing cascade of events causes a mitochondrial dysfunction and rapid decrease in adenosine triphosphate, which leads to the free radical generation and lipid peroxidation (LPO) [1-3].

Oxidative damage has been implicated in various models of acute brain damage and chronic neurodegeneration, including focal ischemic stroke [3-5]. The brain is very susceptible to the damage caused by oxidative stress because of its rapid oxidative metabolic activity, high polyunsaturated fatty acids content, relatively low antioxidant capacity, and inadequate neuronal cells repair activity. Increased levels of reactive oxygen species (ROS) are the major cause of tissue injury after cerebral ischemia, in which inactivation of antioxidant enzymes and consumption of antioxidants such that endogenous antioxidant defense mechanisms fail to protect neurons from oxidative damage [6,7]. Brain tissues are particularly susceptible to oxidative damage; therefore, it is believed that pharmacologic modification of oxidative damage is one of the most promising avenues for stroke therapy.

Experimental models of cerebral ischemia have been developed to improve the understanding of deleterious mechanisms involved in the brain ischemic damage and to study the potential efficiency of prophylactic/therapeutic strategies. Among all the animal models of ischemic stroke, filamentous reversible middle cerebral artery occlusion (MCAO) is one of the most widely used experimental paradigms to induce focal cerebral ischemia [8-10]. The importance of these models lies in preclinical testing of drugs designed for neuroprotection that ultimately may improve functional recovery from stroke.

The molecular mechanism involved in ischemic brain injury is not fully understood; much progress has been made in identifying some signaling pathways such as oxidative stress, excitotoxicity, and inflammation that might be involved in ischemic cell death. Unfortunately, this knowledge has not yet translated into new clinical therapies, and development of neuroprotective agents that are effective clinically remains a high priority.

S-allyl cysteine (SAC) is the most abundant organosulfur compound in garlic extract with potential antioxidant and antiinflammatory properties [11-14]. The therapeutic effects of SAC were assessed in various models of neurodegenerative diseases including stroke [15,16], Alzheimer disease [17,18], and Parkinson disease [19]. The molecular mechanisms of these effects may include protecting neurons against oxidative/nitrosative stress, mitochondrial damage, and subsequent cell death. S-allyl cysteine also reduces edema formation in the ischemic rat brain through the inhibition of LPO [20] and produces neuroprotective effects on the amyloid-beta peptide-induced oxidative damage, and learning deficits [17]. Recently, our research group has investigated and reported the neuroprotective efficacy of SAC in a mouse model of streptozotocin-induced experimental dementia of Alzheimer type [18].

Therefore, the purpose of this study was to examine the effects of SAC administration on neurologic deficits and biomarkers of oxidative stress in rat model of focal cerebral ischemia. We hypothesized that SAC supplementation would ameliorate oxidative damage, improve behavioral activities, and suppress neuronal loss. To test this hypothesis, activities of antioxidant enzymes, LPO, and level of glutathione along with expression of inducible nitric oxide synthase (iNOS) and glial fibrillary acidic protein (GFAP) were assessed. All these actions require further study to elucidate a mechanism in animals before studies in human subjects. Therefore, to understand the mechanism of neuroprotective effect of SAC, we investigated the effect of SAC in rats on various oxidative stress parameters that can be translated to studies on neuroprotection in the human and the applications of SAC in human nutrition.

2. Methods and materials

2.1. Chemical and reagents

S-allyl cysteine was purchased from LGC Prochem, Bangalore, India. Glial fibrillary acidic protein and iNOS antibody were purchased from Chemicon International, Temecula, Calif, and antimouse IgG was purchased from Jackson Immuno Research Laboratories, Inc, West Groove, Pa.

2.2. Animals

Male Wistar rats weighing 250 to 300 g were obtained from the Central Animal House, Jamia Hamdard, New Delhi, India. They were housed in polypropylene cages in air-conditioned room and allowed free access to pellet diet and water *ad libitum*. The animals were used in accordance with the procedure approved by the Animal Ethics Committee of Jamia Hamdard.

2.3. Experimental protocol

To investigate the neuroprotective effects of SAC, we used the rat MCAO model. Animals were divided into 4 groups of 8 animals each. The first group served as sham, and saline was given; in the second group, MCAO was performed, that is, ischemia was induced for 2 hours followed by reperfusion for 22 hours; in the third group, MCAO was performed with addition of treating the rats with SAC (ie, SAC + MCAO group); and the fourth group was sham treated with drug alone, that is, SAC + S group. S-allyl cysteine was dissolved in saline and given in a dose of 100 mg/kg intraperitoneally 30 minutes before the onset of ischemia. Additional injections of 100 mg/kg were administered at 0, 6, and 12 hours of post-