Chapter-IV

S-allyl cysteine mitigates oxidative damage and improves neurologic deficit in a rat model of focal cerebral ischemia
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) (Shah et al., 2010; Khan et al., 2010; Chen et al., 2011).

Oxidative damage has been implicated in various models of acute brain damage and chronic neurodegeneration, including focal ischemic stroke (Allen and Bayraktutan, 2009; Niizuma et al., 2009). 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 (Loh et al., 2010; Sun et al., 2009).

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 (Longa et al., 1989; Takano et al., 1997; Khan et al., 2009). 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 (Maldonado et al., 2003; Garcia et al., 2008; Rojas et al., 2010). The therapeutic effects of SAC were assessed in various models of neurodegenerative diseases including stroke (Kim et al., 2006; Atif et al., 2009), Alzheimer...
disease (Pérez-Severiano et al., 2004; Javed et al., 2011), and Parkinson disease (García et al., 2010). 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 (Numagami et al., 1996) and produces neuroprotective effects on the amyloid-beta peptide-induced oxidative damage, and learning deficits (Pérez-Severiano et al., 2004). 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 (Javed et al., 2011). 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.

**Methods and materials**

**Chemical and reagents**

As described in material and methods, chapter-II.

**Animals and treatments**

As described in material and methods, chapter-II.

**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 (i.e., 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- MCAO. After completion of the reperfusion period, the animals were assessed for neurobehavioral activities and then euthanized with high dose of anesthesia. The hippocampus and frontal cortex were removed from the brain for biochemical estimations.
Induction of transient focal cerebral ischemia (MCAO)
As described in material and methods, chapter-II.

Post-operative care
As described in material and methods, chapter-II.

Behavioral studies
Evaluation of neurologic deficit
As described in material and methods, chapter-II.

Rotarod (motor coordination skill) test
As described in material and methods, chapter-II.

Grip test
As described in material and methods, chapter-II.

Evaluation of ischemic damage by 2, 3, 5-Triphenyltetrazolium chloride staining
As described in material and methods, chapter-II.

Biochemical studies
Tissue preparation for the assays of TBARS, GSH and antioxidant enzymes
After the behavioral studies, the animals were killed, and their brains were removed to dissect out hippocampus and frontal cortex for the biochemical assays (TBARS, GSH, GPx, GR, SOD and Catalase) as described in material and methods, chapter-II.

Histopathologic studies
The brains of each group animals were perfused as described by Khan et al (2010). The brains were removed quickly and kept in the same fresh buffer containing 30% sucrose. The brains were cut into 12-μm-thick coronal sections on a cryostat (Leica, Nußloch, Heidelberg, Germany). Every 10 sections of the cortex region was mounted on glass slides and processed for hematoxylin and eosin staining. As described in material and methods, chapter-II.

Immunohistochemistry for iNOS and GFAP
After 22 h of reperfusion the animals were anesthetized with chloral hydrate (400 mg/ kg, i.p.) and perfused transcardially with 0.9% sodium chloride at 4 °C, followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (PB, pH 7.4). The brain was removed, and processed as describe in material and methods, chapter-II.

Estimation of protein concentration
Protein was determined by the method of Lowry et al (1951) using bovine serum albumin as standard.

Statistical analyses
As described in material and methods, chapter-II.
Results

Effect of SAC on behavioral output

Rotarod
As measured by rotarod, a significant decrease in muscular coordination skill was observed in MCAO group as compared with the sham group animals. Animals treated with SAC in the SAC + MCAO group afforded a significant (p<0.01) protection in muscular coordination skill, as compared with MCAO group animals; however, no significant differences were observed between the sham and the SAC-treated sham group (SAC + S) (Fig. 1A).

Grip strength
The mean score was decreased significantly (p<0.01) in the MCAO group as compared with the sham group rats. S-allyl cysteine treatment has increased the mean scores significantly (p<0.05) in the SAC + MCAO group as compared with the MCAO group (Fig. 1B). However, no significant alteration in grip strength was observed in the SAC-treated sham (SAC + S) group as compared with the sham group.

Effect of SAC on neurologic deficits
Middle cerebral artery occlusion rats have poor neurologic function as compared with the sham group. Treatment with SAC improved neurologic deficits in the SAC + MCAO group animals as compared with the MCAO group rats (Fig. 1C). No significant alteration was observed in SAC treated sham group (SAC + S) as compared with the sham group.

Fig. 1 A, Effect of SAC treatment on muscular coordination skill in MCAO rats. A, Middle cerebral artery occlusion leads a significant depletion in motor coordination as compared with sham group and significantly recovered in SAC-treated MCAO group (SAC + MCAO) as compared with MCAO group. Values are expressed as means ± S.E.M for 8 animals. *p<0.001, MCAO vs sham; #p<0.01, SAC + MCAO vs MCAO. B, The grip strength was decreased significantly in the MCAO group animals as compared with the sham group animals. Treating the animals with SAC followed by MCAO has protected grip strength as compared with MCAO group. Values are expressed as means ± SEM of 8 animals. *p<0.01, MCAO vs sham; #p<0.05, SAC + MCAO vs MCAO.
Effect of SAC on TTC stain and infarction volume

We hypothesized that SAC plays a protective role in stroke. Indeed, the MCAO group rats have shown a significantly increased infarct volume as compared with sham. 2,3,5-Triphenyltetrazolium chloride staining of MCAO brain sections showed reproducible and readily detectable lesions in the areas that are supplied by the MCA after 22 hours of reperfusion (Fig. 2A). S-allyl cysteine treatment has reduced the infarct volume significantly (p<0.05) as compared with the MCAO group (Fig. 2B).

Fig. 2A, Effect of SAC treatment on brain infarct size by TTC stain after MCAO for 2 hours and reperfusion of 22 h. A, Representative photographs of brain sections stained with 0.1% TTC; B, measurement of infarct volumes of the MCAO and SAC + MCAO groups are presented. The MCAO group produced a significant lesion over the sham group (figure not shown). However, the SAC + MCAO group showed a significant (*p<0.05) reduction in tissue damage as compared with the MCAO group.
Effect of SAC on endogenous antioxidant system

S-allyl cysteine treatment decreased the TBARS contents in hippocampus and frontal cortex

The effect of SAC on TBARS content was measured to demonstrate the oxidative damage to lipids in hippocampus and frontal cortex of MCAO group. A significantly increased (*p<0.01) TBARS content was observed in the MCAO group animals as compared with sham group. Rats of the SAC + MCAO group exhibited significant attenuation (#p<0.05) in TBARS content as compared with the MCAO group (Fig. 3). The SAC-alone-treated group showed no significant changes in TBARS content as compared with the sham group.

S-allyl cysteine treatment restored the GSH level in hippocampus and frontal cortex

Protective effect of SAC on GSH level in hippocampus and frontal cortex was observed. The level of GSH was depleted significantly in the hippocampus (*p<0.05) and frontal cortex (**p<0.01) in the MCAO group as compared with the sham group. S-allyl cysteine treatment has protected its level significantly (P<0.05) in the SAC + MCAO group as compared with the MCAO group. The SAC-alone-treated group (SAC + S) exhibited no significant changes in GSH level as compared with the sham group (Fig. 4).
S-allyl cysteine treatment attenuated the activities of antioxidant enzymes in the hippocampus and frontal cortex

The activities of antioxidant enzymes (GPx, GR, SOD, and catalase) were decreased significantly in hippocampus and frontal cortex of the MCAO group animals as compared with the sham group animals, and their activities were restored significantly in the frontal cortex as well as in the hippocampus of the SAC-treated MCAO group (SAC + MCAO) animals. No significant change was observed in the SAC-treated sham group (SAC + S) animals as compared with the sham group animals (Tables 1 and 2).

Fig. 4. Effect of SAC treatment on GSH level in the hippocampus and frontal cortex. Reduced glutathione level was significantly decreased in hippocampus (*p<0.05) and frontal cortex (**) in the MCAO group rats as compared with the sham group. S-allyl cysteine treatment has significantly (#p<0.05) increased the level of GSH in the SAC+MCAO group as compared with the MCAO group. Values are expressed as means ± SEM (n = 8).
Table 1. Activities of antioxidant enzymes (GPx, GR, G6-PD, GST and SOD) in hippocampus of MCAO rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>MCAO</th>
<th>SAC+MCAO</th>
<th>SAC+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>289.34 ± 10.06</td>
<td>172.31±6.15*</td>
<td>250.81±8.24#</td>
<td>289.84±9.54</td>
</tr>
<tr>
<td>GR (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>219.54 ± 9.58</td>
<td>133.46 ± 6.04*</td>
<td>190.06 ± 10.56#</td>
<td>212.52 ± 6.96</td>
</tr>
<tr>
<td>SOD (nmol of epinephrine protected from oxidation/ min/ mg protein)</td>
<td>422.9 ± 31.63</td>
<td>244.4 ± 10.81*</td>
<td>331.09 ± 15.59#</td>
<td>420.08 ± 24.05</td>
</tr>
<tr>
<td>CAT (nmol of H2O2 consumed/ min/ mg/ protein)</td>
<td>8.65 ± 0.79</td>
<td>3.63 ± 0.33*</td>
<td>5.92 ± 0.31#</td>
<td>8.83 ± 0.59</td>
</tr>
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MCAO leads to significant (*p<0.05) alterations on the activities of antioxidant enzymes (GPx, GR, SOD, and CAT) in hippocampus in the MCAO group animals as compared with the sham group animals. Administration of SAC has significantly (#p<0.01) attenuated the activity of these enzymes in the SAC + MCAO group animals as compared with the MCAO group animals. Values in parentheses show the percentage increase or decrease with respect to their control. Values are expressed as means ± SEM of n = 8 animals. Values in parentheses show the percentage increase or decrease with respect to their control.

Table 2. Activities of antioxidant enzymes (GPx, GR, G6-PD, GST and SOD) in frontal cortex of MCAO rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sham</th>
<th>MCAO</th>
<th>SAC+MCAO</th>
<th>SAC+S</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPx (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>297.01 ± 11.91</td>
<td>174.23 ± 10.84*</td>
<td>253.98 ± 16.18#</td>
<td>283.59 ± 12.37</td>
</tr>
<tr>
<td>GR (nmol of NADPH oxidized/ min/ mg protein)</td>
<td>229.85 ± 10.25</td>
<td>134.75 ± 7.26*</td>
<td>170.26 ± 7.58#</td>
<td>230.71 ± 12.10</td>
</tr>
<tr>
<td>SOD (nmol of epinephrine protected from oxidation/ min/ mg protein)</td>
<td>433.40 ± 18.98</td>
<td>248.10 ± 14.26*</td>
<td>351.22 ± 13.87#</td>
<td>434.15 ± 28.34</td>
</tr>
<tr>
<td>CAT (nmol of H2O2 consumed/ min/ mg/ protein)</td>
<td>9.85 ± 0.70</td>
<td>3.85 ± 0.38*</td>
<td>5.99 ± 0.43#</td>
<td>9.82 ± 0.56</td>
</tr>
</tbody>
</table>

MCAO leads to significant alterations on the activities of antioxidant enzymes (GPx, GR, SOD and CAT) in hippocampus in MCAO group animals as compared with the sham group animals. Administration of SAC has significantly attenuated the activity of these enzymes in SAC+MCAO group animals as compared with the MCAO group animals. Values in parentheses show the percentage increase or decrease with respect to their control. Values are expressed as means ± S.E.M of n = 8 animals. Values in parentheses show the percentage increase or decrease with respect to their control.

Morphological changes

Histopathologic changes in neuron after ischemia/reperfusion injury were investigated by hematoxylin-eosin staining. The sections of the sham group showed normal cell with no pathologic change, whereas the sections of the MCAO group showed a focus of brain damage.
with neuronal loss and presence of numerous vacuolated spaces. Intact neurons were absent in that area. The corresponding area in the sections from the SAC + MCAO group showed partial neuronal loss with presence of intact neurons in between the vacuolated spaces. S-allyl cysteine treatment ameliorated neuronal abnormalities in the SAC + MCAO group as compared with the MCAO group animals (Fig. 5).

Fig. 5. Effect of SAC treatment on hematoxylin and eosin staining in the brain sections of the sham, MCAO, and SAC + MCAO groups. A, Cortical area of sham group animal showed uniform distribution of neurons. Normal neurons with the characteristic conical outlines with no abnormal features are seen. B, Tissues around infarcted area in the MCAO group show a focal area of vacuolation and neuronal loss. C, The SAC + MCAO group rats show partial neuronal loss. Original magnification ×20.

**Effect of SAC treatment on GFAP and iNOS expression in MCAO rats**

The activation of astrocyte up-regulation is associated with neuronal cell death in cerebral ischemia. Glial fibrillary acidic protein expression was found to be remarkably high in the ischemic hemisphere of the MCAO group (Fig. 6). A noticeable reduction in GFAP expression was observed in the SAC-treated group as compared with the MCAO group. Expression of GFAP was seen to be very scarce in the sham group animals. S-allyl cysteine treatment did not show any remarkable effects in the SAC + S group as compared with the sham group (data not shown). Increased staining of iNOS was observed in the ischemic hemisphere of the MCAO group, which was well attenuated with the treatment of SAC. However, iNOS expression was almost negligible in the sham group. S-allyl cysteine treatment did not show any remarkable effects in the SAC + S group as compared with the sham group (data not shown).
Discussion
In the present study, the effects of SAC after focal cerebral ischemia/reperfusion injury in rats were investigated. We demonstrated that the treatment with SAC significantly improved the functional outcome, attenuated the redox imbalance via ameliorating oxidative damage, and suppressed the neuronal loss because of its potential antioxidant property. It is well documented that MCAO rodent model is an appropriate animal model used for the study of stroke (Sun et al., 2009; Khan et al., 2009). The MCAO model with reperfusion that recapitulates many features of the stroke in humans was used because the MCA, which is the specific occlusion site in this model, is the most commonly affected vessel in both embolic and thrombotic strokes in humans (Khan et al., 2010; Longa et al., 1989; Khan et al., 2009; Saleem et al., 2006). It is well documented that MCAO results in behavioral, neurochemical, and histologic alterations in rat brain and generation of free radicals has been implicated to be one of the main contributing factors. Our results suggest that SAC offers better neuroprotection and could shield the brain.

Fig. 6. Representative coronal brain sections of the sham, MCAO, and SAC + MCAO group rats stained for GFAP and iNOS. Cortical neuronal area of brain from the MCAO group (B) animals showing significant increase in GFAP and iNOS expression as compared with the sham group (A), and this expression was decreased in the SAC+MCAO group (C) as compared with MCAO group. Original magnification ×20.
from the deleterious effects of stroke. Neuroprotective potential of SAC suggests that it is a powerful antioxidant, corroborating previous studies (García et al., 2008; Atif et al., 2009; Javed et al., 2011).

The behavioral effects are intertwined with the degree of neuronal dysfunction (Schwarting et al., 1991). Functional deficits are common neurologic sequel in patients with brain injuries and animal models of cerebral ischemia. Furthermore, free radicals are always known to play vital role in neurobehavioral deficits in experimental models through oxidative stress, so the poor neurobehavioral outcome in MCAO group rats might be attributed to oxidative stress-induced free radicals. Earlier studies have shown an improvement in various behavioral outputs as a result of antioxidant treatment (Khan et al., 2009; Zhao et al., 2008; Xue et al., 2010). In line with above studies, we observed that the SAC-treated MCAO group has shown significant improvement in behavioral and functional outcome in terms of rotarod task, grip strength, and neurologic scores reflecting the antioxidant potential of SAC. Our findings correlate well with the earlier studies carried out by us and others where motor deficits have been attenuated by treatment with antioxidants (Khan et al., 2009; Zhao et al., 2008; Xue et al., 2010).

Infarction volume in the brain is an important determinant in assessing the consequences of ischemic stroke, which leads to severe neuronal damage in the different brain parts with subsequent neurologic impairment. 2, 3, 5-Triphenyltetrazolium chloride methods have been used to detect themorphological features of infarct tissue after ischemic injury (Liszczak et al., 1984; Bederson et al., 1986). In the present study, the MCAO group showed a prominent infarct size along with significantly altered behavioral outputs. S-allyl cysteine treatment not only reduced the infarct size but also improved behavioral deficits in the MCAO group treated with SAC. Animal experiments have indicated that SAC administered after cerebral ischemia are effective in reducing infarct volume and lead to improvements in neurologic outcome (Kim et al., 2006; Numagami et al., 1996).

The brain has multiple sources of ROS (Faraci et al., 2006) and a large oxidative ability, but its capacity to fight against oxidative stress is limited (Mantha et al., 2006). Oxidative stress has an important role in the pathogenesis of ischemic brain damage. Ischemia induces an imbalance of endogenous oxidants and antioxidants and overproduction of toxic free radicals. Reperfusion also comes with robust production of ROS and nitrogen species that potentiate initial brain damage caused by ischemia. Evidence has accumulated showing that the massive generation of reactive free radicals during reperfusion and the resulting formation of peroxynitrite cause LPO, protein oxidation, and DNA damage (Loh et al., 2010; Broughton et al., 2009; Ahmad et al., 2011).
Middle cerebral artery occlusion leads to highly reactive ROS production increasing LPO, which leads to cellular disintegration and neuronal loss. Lipid peroxides and hydroperoxides cause secondary injury by further generating relatively more stable and diffusible cytotoxic agents such as malondialdehyde and 4-hydroxy-trans-2-nonenal, respectively, and amplify oxidative cascade (Khan et al., 2009; Chang et al., 2009). They react avidly with cellular nucleophiles such as glutathione (GSH) and cause continuous decrease in its level through increased oxidant content or protein modification. Reduced glutathione is a well-known antioxidant that is synthesized in the cytoplasm and is present in higher concentrations in the mitochondrial matrix. The low levels of GSH may be directly related to increased ROS, lipid peroxides, and highly reactive hydroxyl radicals (Loh et al., 2010; Khan et al., 2009; Ahmad et al., 2005). Furthermore, GSH plays a crucial and critical role in the regulation of expression of several antiinflammatory genes. Thus, GSH inhibition in cerebral ischemia would increase the susceptibility of plasma membranes toward peroxide attacks. However, the main cause of GSH loss during oxidative stress in brain ischemia is the formation of protein glutathione mixed disulphide (PrSSG) and loss of thiol proteins (Reed, 1990). The loss of GSH and formation of PrSSG in the brain results in the various membrane dysfunction, such as inhibition on Na+ K+ adenosine triphosphatase activity. There are several reports about the modulatory effect of SAC on LPO, glutathione, and antioxidant enzymes after brain injury (Atif et al., 2009; Pérez-Severiano et al., 2004; Javed et al., 2011). In agreement with these finding, we also found that SAC significantly reduced the TBARS level along with increased glutathione level and activities of antioxidant enzymes. Superoxide dismutase scavenges superoxide radicals by catalyzing the conversion of 2 of these radicals into hydrogen peroxide and molecular oxygen (Freeman et al., 1982). The hydrogen peroxide formed by SOD and by other processes is scavenged by GPx and CAT, a ubiquitous protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen.

Glutathione reductase and GPx are known to be inactivated by oxidant radicals, as observed in the present study and some earlier studies (Sharma and Gupta, 2001; Kumar and Gupta, 2003). Glutathione reductase is found in many tissues, and it enables the cell to sustain adequate levels of cellular GSH. Reduced glutathione is a substrate for GPx, which plays a predominant role in removing excess free radicals and hydroperoxides (Imam and Ali, 2000). Previous studies confirm that SAC is a powerful antioxidant, which scavenges free radical–induced damages (Jungsook, 2006). The present data encompass the role of oxidative radicals in the depletion of glutathione and its dependent enzymes, which were protected significantly by the treatment of SAC, reflecting its potential antioxidant potential.
Besides defending against oxidant stress, another existing and encouraging finding is that SAC significantly attenuated histologic changes, that is, it caused minimal glial cell infiltration, less neural damage with small vacuolated space, along with presence of intact neuron in the neuronal tissue as compared with ischemic cortical neuronal loss.

The inducible form of NOS (iNOS) and astrogliosis have been implicated as an important mediator of inflammatory responses during ischemia and reperfusion (Basu et al., 2002; Samdani 1997; Shen et al., 2007; Danielisova et al., 2011). The analysis of reactive astrocytosis and Proinflammatory enzymes iNOS around the lesion also revealed a significant effect of SAC. The administration of SAC significantly reduced the GFAP and iNOS expression in the cortical region. These results suggest that SAC administration after MCAO significantly reduces tissue damage at the site of injury. It has been reported that blockade of iNOS expression by pharmacologic agents is associated with inhibition of stroke severity (Yamakawa et al., 2003; Hsu et al., 2007). Together, our data and similar evidence in MCAO model of cerebral ischemia further support the neuroprotective potential of SAC. Additional research is needed to further investigate this hypothesis. A limitation of this study is that we have evaluated the neuroprotective effect of SAC at 1 time point. Further investigation into the neuroprotective role of SAC is needed at different time point after MCAO.

In conclusion, the data obtained in this study support our hypothesis that the repeated administration of SAC suppresses the progression of ischemic damage through antioxidant and antiinflammatory properties. All of these results provide greater insight on the potential use of SAC in humans.

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