Dietary S-Allyl-L-cysteine Reduces Mortality with Decreased Incidence of Stroke and Behavioral Changes in Stroke-Prone Spontaneously Hypertensive Rats

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S-Allyl-L-cysteine (SAC), an active organosulfur compound derived from garlic, was found to reduce mortality with lesser incidence of stroke and also to lower the overall stroke-related behavioral score in stroke-prone spontaneously hypertensive (SHRSP) rats by dietary administration. Consequently, the anti-stroke effect of dietary SAC was demonstrated in SHRSP rats.

Key words: S-allyl-L-cysteine; stroke-prone spontaneously hypertensive rat; stroke onset; mortality; stroke-related behavioral change

Numerous sulfur-containing compounds derived from garlic (Allium sativum L.) have been reported to have various biological activities. Recently, S-allyl-L-cysteine (SAC), one of the active water-soluble compounds in garlic products, has been found to have neuroprotective effects in various in vitro and in vivo models of neurodegenerative diseases. Particularly, intraperitoneal administration of SAC reduced edema formation in ischemic rat brain through inhibition of lipid peroxidation. Furthermore, SAC also ameliorated learning deficits in senescence-accelerated mice, evoked neurotrophic action and protected amyloid-β-induced neurotoxicity in hippocampal cultures.

Several studies have shown that improvements in nutritional factors are effective to prevent cerebral stroke and lengthen the life span in stroke-prone spontaneously hypertensive (SHRSP) rats. Although SAC, known as a neuroprotectant, may possibly prevent cerebral stroke, the effect of dietary SAC on SHRSP is unknown. In this study, we investigated the effect of dietary SAC on stroke onset time, incidence, mortality, stroke-related behavioral changes, and blood pressure in SHRSP in comparison with the normotensive genetic control, Wistar-Kyoto (WKY) rats.

Six-week-old male SHRSP/Izm and age-matched WKY/Izm rats as normotensive genetic controls, were purchased from the Japan SLC (Shizuoka, Japan). The animals were allowed 2 weeks for acclimatization and then were divided into three groups: (1) an age-matched normotensive WKY control group (n = 10), (2) an SHRSP control group (n = 9), and (3) an SHRSP + SAC group (n = 9). All groups were fed a stroke-prone diet (Funabashi SP, Chiba, Japan) supplemented with (for the SHRSP + SAC group) or without (for the WKY and SHRSP control groups) 0.5% (w/w) SAC (> 99%, TCI Chemical, Tokyo). To bring on early development of stroke, all groups were given drinking water containing 1% (w/v) NaCl for 28 d. They were individually housed in an area having 22 ± 2°C and 55 ± 5% relative humidity with a light/dark cycle of 12 h. Body weight, food intake, and water intake were monitored on a daily basis. Blood pressure was measured weekly by tail-cuff plethysmography (Letica Scientific Instruments LE5002, Barcelona, Spain) without anesthesia. All experiments were carried out in accordance with the Guidelines for Animal Experimentation (Korean Association for Animal Laboratory Science, 2001) and the protocol was approved by our Institutional Animal Care Committee.

The onset of stroke was judged by sudden weight loss, decreased food consumption, and increased fluid intake. Each rat was evaluated by a double-blind behavioral examination. The parameters tested were rated as either 0 (normal) or 1 to 3 depending on the severity of incapacitation. The stroke-related behavioral score consisted of the sum total (maximum 12) assigned to disabilities such as seizure, paralysis, loss of body symmetry, loss of motor coordination, gait response, and balance, as reported previously. All rats were examined histologically following death and the cause of

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Note
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death was repeatedly confirmed to be stroke.\textsuperscript{17} Statistical differences were determined by the Mann-Whitney test or the Chi-squared test, and \( P < 0.05 \) was considered significant (SAS Institute, Cary, NC).

The results shown in Fig. 1 present the effect of dietary SAC supplementation on stroke onset time, stroke incidence, the mortality rate, and stroke-related behavioral scores in the age-matched normotensive WKY control, SHRSP control, and SHRSP + SAC groups during the 28-d diet period. Dietary SAC supplementation significantly reduced stroke incidence, by about 22\%, as compared to the incidence occurring in the SHRSP control group (Fig. 1B) \( (X^2 = 26.96, P = 0.0001; X^2 = 7.38, P = 0.025) \). No mortality was observed in the SHRSP + SAC group, while 33\% mortality (three of nine animals) was observed in the SHRSP control group within the 28-d diet period (Fig. 1C) \( (X^2 = 7.38, P = 0.025) \). In contrast, stroke onset times, expressed as the number of days taken to manifest the first stroke sign from the start of a stroke-prone diet and salt loading, was not significantly different between the SHRSP control and SHRSP + SAC groups (Fig. 1A). One possible reason for this discrepancy between stroke incidence and stroke onset time is that the remaining rats without stroke signs in the SHRSP + SAC group could not be included to determine stroke onset time, as stated above. The SHRSP + SAC group tended to have a lower stroke-related behavioral score than the SHRSP control. Specifically, greater improvements in the SHRSP + SAC group were found in paralysis, gait response, motor coordination, and body symmetry. However, due to the low incidence of behavioral changes in the SHRSP + SAC group for 26 d (two of nine animals) and intra-variation within the groups, statistical significance could not be pinpointed. Significance was found only on the 28th d (Fig. 1D). SHRSP + SAC rats appeared to be more vital and active than SHRSP control ones, which were sedated. These findings suggest that dietary SAC administration can have been effective in attenuating neuronal injury due to stroke in the SHRSP group, in spite of genetic predisposition and severe interference in homeostasis by salt-loading.

At present, we do not know the exact mechanism of the anti-stroke effect of SAC. SAC has been shown to inhibit oxidative damage by scavenging reactive oxygen or nitrogen species such as superoxide anions, hydroxyl radicals, and nitrogen monoxide, or by modulating their production.\textsuperscript{18,19} Recently, we found that SAC exerted its...
neuroprotective effect in cerebral ischemic insult by scavenging peroxynitrite and by inhibiting the extracellular signal-regulated kinase signaling pathway activated during initial hypoxic/ischemic insults (Kim, Lee, Chang, Chun and Kim, unpublished). In this study, dietary SAC administration did not change the blood pressure of SHRSP rats (data not shown). This implies that the anti-stroke effect of SAC does not result simply from its modulation of the blood pressure of SHRSP. Further experiments are necessary to delineate the exact mechanism for the anti-stroke effect of SAC in SHRSP.

These findings indicate that dietary intake of SAC might play a role in attenuating the incidence and behavioral dysfunction of a stroke, which in turn can contribute to reduced mortality rates, possibly independent of blood pressure-lowering, in SHRSP. To our knowledge, this is the first demonstration of a reduction of stroke incidence by dietary SAC administration. The present findings perhaps provide good grounds for the wider use of SAC as a dietary supplement in conditions associated with stroke.

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References