

The effect of sesamol on rats with ischemic stroke

BO YOUNG HONG, MD, PhD¹⁾, JOON SUNG KIM, MD, PhD¹⁾, KYOUNG BO LEE, PT, PhD¹⁾,
SEONG HOON LIM, MD, PhD^{1)*}

¹⁾ Department of Rehabilitation Medicine, St. Vincent's Hospital, College of Medicine, The Catholic University of Korea: 93 Jungbu-daero, Paldal-gu, Suwon 442-723, Republic of Korea

Abstract. [Purpose] Although previous studies have demonstrated several effects of sesamol on neurological diseases, its effects on ischemic stroke are unclear. We evaluated the direct effects of sesamol on infarcts and efficacy in terms of functional improvement in rats with transient middle cerebral artery occlusion (MCAO). [Subjects and Methods] Male Sprague Dawley rats ($n = 30$) were randomly divided into two groups: an MCAO with sesamol group and an MCAO group. MCAO was induced for 2 h, and sesamol was administered in the treatment group just after reperfusion. Infarct size was calculated 5 days after MCAO. Efficacy in function was assessed using a modified sticky-tape test (MST) and percent weight borne on the paretic leg during 5 days. [Results] Infarct volumes did not differ significantly between the two MCAO groups. The values of MST did not differ between the two MCAO groups. Based on the values of percent weight borne on the paretic leg, function of the hindlimb in the MCAO with sesamol group was significantly better than in the MCAO group throughout the experimental period. [Conclusion] These results demonstrate that sesamol induced functional improvements during 5 days after MCAO, and could be a useful addition to the therapeutic regimen for the treatment of ischemic stroke.

Key words: Sesamol, Middle cerebral artery occlusion, Ischemic stroke

(This article was submitted Jan. 19, 2015, and was accepted Feb. 14, 2015)

INTRODUCTION

Several million people per year suffer strokes, and there is still no established treatment for lessening the neurological damage. Many studies have examined ways to reduce neuronal damage after ischemic stroke, but the results are unclear. For safety and feasibility, finding candidate substances for neuroprotection in food materials has been investigated in many studies^{1, 2)}.

Sesamol (5-hydroxy-1,3-benzodioxole or 3,4-methylenedioxyphenol) is a major constituent of sesame seed oil (obtained from *Sesamum indicum*). Today, sesamol is known as a potent antioxidant that may have neuroprotective effects^{3, 4)}. Recent studies have reported the beneficial effects of sesamol on cognitive impairment and depression^{3, 4)}. However, its effects on ischemic stroke are not yet clear. The proper experimental duration when studying ischemic stroke is also not clear, and it has ranged from 24 h to 3 weeks^{2, 5–7)}. In a previous study on diabetes-associated cognitive decline (in terms of performance in spatial memory tasks), the efficacy of sesamol was demonstrated within 5 days³⁾. Thus, we investigated the effects of sesamol on ischemic stroke within 5 days after ischemia/reperfusion.

We assessed whether sesamol had a positive effect on

functional improvement in rats whose middle cerebral arteries were transiently occluded. Specifically, we examined whether treatment would improve upper and lower limb function within 5 days and also reduce the neuronal lesion.

SUBJECTS AND METHODS

In total, 30 male Sprague Dawley rats (12 weeks old, 363 ± 21 g) were purchased from Koatech Inc. (Pyungtaeck, Republic of Korea). All experimental procedures were approved by the Animal Experimental Committee of the Catholic University of Korea. The rats were randomly divided into two groups: stroke with sesamol treatment (MCAO with sesamol, $n = 15$) and stroke with no treatment (MCAO, $n = 15$).

Rats were anesthetized with an intraperitoneal (i.p.) injection of Zoletil (tiletamine/zolazepam, 0.2 mL/kg, intraperitoneal injection) and xylazine hydrochloride (4 mg/kg), and left middle cerebral artery occlusion was induced using the monofilament thread method^{6, 8–10)}. After 2 h of occlusion, reperfusion was performed in the MCAO groups, with careful removal of the monofilament¹¹⁾. Immediately after reperfusion, sesamol (8 mg/kg, oral gavage; Sigma, St. Louis, MO, USA) was administered to the treatment group^{1, 2)}. The MCAO with sesamol and MCAO groups received an equivalent amount of saline solution. Body temperature was measured by inserting a thermometric probe into the rectum of the rat, and the temperature was maintained at 37.5 ± 0.5 °C during the operation using a heating pad⁵⁾.

Five days after reperfusion, all rats were re-anesthetized with Zoletil (0.2 mL/kg, i.p.) and decapitated. Brains were removed carefully and sectioned coronally at 2 mm inter-

*Corresponding author. Seong Hoon Lim (E-mail: seonghoon@catholic.ac.kr)

vals, beginning at the frontal pole. The coronal sections were stained with 2% 2,3,5-triphenyltetrazolium chloride (TTC) in phosphate-buffered saline, followed by fixation with formalin rinsing with saline¹². A calibrated image of the posterior surface of each slice was obtained (NIH Image), and the infarct areas were summed to determine the lesion volumes. The lesion volume is presented as a volume percentage of the lesion compared with the contralateral hemisphere, to remove any impact of edema^{2,7}.

All tests were carried out the day before ischemia was induced and on days 1, 3, and 5 after ischemia. The tests were performed at predetermined times, to exclude behavioral changes associated with circadian rhythms. A 5-min resting period was allowed between each test. Functional assessments were performed in a random order, to reduce potential bias.

To assess sensorimotor function in the forelimb, we used a modified sticky-tape (MST) test^{2, 13, 14}. A sleeve was created using a piece of yellow paper tape (3.0 cm long and 1.0 cm wide; Write-On Label Tape; Bel-Art Products, Pequannock, NJ, USA), which was subsequently wrapped around the forepaw so that the tape attached to itself and allowed the toes to protrude slightly from the sleeve. When applied correctly, the tape sleeve could not be removed. Healthy rats will vigorously attempt to remove the sleeve by pulling at the tape with their mouth and/or brushing the tape with their contralateral paw. Each rat was placed in its cage and observed for 30 s. Two timers were started: the first ran without interruption, and the second was turned on only while the animal attempted to remove the tape sleeve. The contralateral and ipsilateral limbs were tested separately, and the results were expressed as the ratio of the MST values for the left (unaffected) forelimb to those for the right. The test was repeated three times per test day, and the best two scores of the day were averaged.

To assess sensorimotor function in the hindlimb, we used the percentage of weight borne (PWB) on the paretic hindlimb with a weight-bearing apparatus (Linton Incapacitance Tester; Stoelting Co., Wood Dale, IL, USA)^{2, 11}. Three sets of readings (nine measurements) were taken per rat; mean values were calculated and used in the analysis.

All data are presented as means \pm SDs. Differences in infarct volume, MST values, and PWB between the two MCAO groups were evaluated using the Mann-Whitney U-test. All tests were two-tailed, and p values ≤ 0.05 were deemed to indicate statistical significance.

RESULTS

There were originally 15 rats in each group. However, during the experiments, a total of 15 rats died, leaving eight rats in the MCAO with sesamol group (seven deaths) and seven rats in the MCAO group (eight deaths). Of the rats that died, nine did not recover after being subjected to MCAO and died after the termination of anesthesia. The remaining six rats expired within 3 days. Postmortem evaluations revealed extensive infarcts in the left hemisphere with signs of cerebral edema in four rats, and there was evidence of intracranial hemorrhage in four rats. In the remaining seven rats, we did not determine the causes of death by postmor-

Table 1. Volume percentage of the lesion compared with the contralateral hemisphere on day 5 after ischemia in each group

	MCAO with sesamol	MCAO
Total volume (%)	8.04 \pm 2.21	7.96 \pm 3.01
Volume of cortex (%)	4.17 \pm 1.25	3.96 \pm 0.94
Volume of striatum (%)	3.87 \pm 1.10	4.01 \pm 2.20

Values are given as the mean \pm SD.

Table 2. Changes in values in the modified sticky-tape test after MCAO

	MCAO with sesamol	MCAO
Preoperative	1.01 \pm 0.02	1.04 \pm 0.03
Day 1	0.83 \pm 0.16	0.85 \pm 0.23
Day 3	0.75 \pm 0.19	0.81 \pm 0.15
Day 5	0.80 \pm 0.13	0.83 \pm 0.17

The data presented are the ratios of the values for the left (unaffected) forelimb to the right (affected) forelimb. Values are given as the mean \pm SD. MCAO: middle cerebral artery occlusion.

Table 3. Changes in percent weight borne on the paretic hindlimb after MCAO

	MCAO with Sesamol	MCAO
Preoperative (%)	50.13 \pm 0.70	50.65 \pm 1.08
Day 1 (%)	47.60 \pm 1.19*	46.30 \pm 1.60
Day 3 (%)	49.24 \pm 0.95*	47.01 \pm 2.25
Day 5 (%)	48.62 \pm 1.25*	47.15 \pm 1.23

Values are given as the mean \pm SD. MCAO: middle cerebral artery occlusion.

* $p < 0.05$, MCAO group compared with the MCAO + sesamol group.

tem evaluations. These 15 rats were excluded from the final analysis.

The infarct volumes were not different between the two groups (Table 1). The differences in total infarct volume and the infarct volumes of the cortex, and striatum, among the groups were not significant ($p > 0.05$).

No significant differences were found between the groups at baseline or on days 1, 3, or 5 in terms of MST in the forelimbs (Table 2, $p > 0.05$). There were no significant differences in baseline PWB between the groups. After MCAO, the values were significantly lower in the MCAO group than in the MCAO with sesamol group throughout the experimental period (Table 3, $p < 0.05$).

DISCUSSION

We investigated the effects of sesamol on functional recovery. To the best of our knowledge, this is the first reported study to evaluate the effect of sesamol on rats with

MCAO. To demonstrate a positive effect of sesamol on functional improvement in MCAO rat models, we used an optimal dosage based on several past studies^{5, 15}. Evaluation of function was performed using two methods suggested in previous studies^{11, 13, 14}. Our results revealed functional effectiveness in the hindlimb within 5 days post ischemia, consistent with previous studies on the neuroprotective and cognitive-enhancing effects of sesamol^{3, 4, 16}.

Sesamol is a phenolic derivative with a methylenedioxy group; it is a potent antioxidant with chemopreventative, antimutagenic, and anti-hepatotoxic properties^{17, 18}. Sesamol induces increases in the activities of antioxidant enzymes, and its free-radical scavenging properties protects lymphocytes¹⁶. Thus, we hypothesized that as a free-radical scavenger, sesamol may induce a reduction in infarct size and functional improvement by reducing reperfusion injury. A recent study suggested this, reporting that sesamol improved spatial memory acquisition in diabetes-associated cognitive decline and had antidepressant-like effects in chronically stressed mice^{3, 4}. Our results showed functional effectiveness in the hindlimb within 5 days post ischemia; however, we did not find any effect on infarct size or forelimb function.

Our study had several limitations. First, the amount of sesamol may not have been sufficient to see an effect in the forelimb or the infarct itself. Other doses of sesamol will be used to assess other effects in ischemic stroke. Second, the duration of our study was not sufficient to see late effects in chronic stages of ischemic stroke. In future experiments, we will examine effects in chronic ischemic stroke. Third, because of the high rate of mortality, the number of animals in our study was small. Future studies will evaluate more animals, and examine the intracellular pathways involving sesamol.

To date, no substance has been shown clinically to result in functional improvement after cerebral infarction. Furthermore, many of the therapeutic interventions assessed in animal studies have not worked clinically¹⁹. Our study of sesamol, derived from sesame seed oil, may be of practical use in the clinical field in that it may be easily applicable as a therapeutic intervention in stroke patients, and its effects can easily be measured in terms of functional outcome. We found a positive effect on hindlimb function in rats treated with sesamol. Sesamol or sesame seed oil intake may be a useful addition to the treatment regimen during the acute rehabilitation period. However, additional clinical research and epidemiological studies are necessary to assess its efficacy for clinical use.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the Catholic Medical Center Research Foundation made in the program year of 2012. No commercial party having a direct financial interest in the results of the research sup-

porting this article has or will confer a benefit upon the authors or upon any organization with which the authors are associated.

REFERENCES

- 1) Lee S, Suh S, Kim S: Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. *Neurosci Lett*, 2000, 287: 191–194. [[Medline](#)] [[CrossRef](#)]
- 2) Lim SH, Kim HS, Kim YK, et al.: The functional effect of epigallocatechin gallate on ischemic stroke in rats. *Acta Neurobiol Exp (Warsz)*, 2010, 70: 40–46. [[Medline](#)]
- 3) Kuhad A, Chopra K: Effect of sesamol on diabetes-associated cognitive decline in rats. *Exp Brain Res*, 2008, 185: 411–420. [[Medline](#)] [[CrossRef](#)]
- 4) Kumar B, Kuhad A, Chopra K: Neuropsychopharmacological effect of sesamol in unpredictable chronic mild stress model of depression: behavioral and biochemical evidences. *Psychopharmacology (Berl)*, 2011, 214: 819–828. [[Medline](#)] [[CrossRef](#)]
- 5) Choi YB, Kim YI, Lee KS, et al.: Protective effect of epigallocatechin gallate on brain damage after transient middle cerebral artery occlusion in rats. *Brain Res*, 2004, 1019: 47–54. [[Medline](#)] [[CrossRef](#)]
- 6) Kim G, Kim E: Effects of treadmill training on limb motor function and acetylcholinesterase activity in rats with stroke. *J Phys Ther Sci*, 2013, 25: 1227–1230. [[Medline](#)] [[CrossRef](#)]
- 7) Zhang Y, Jin Y, Behr MJ, et al.: Behavioral and histological neuroprotection by tamoxifen after reversible focal cerebral ischemia. *Exp Neurol*, 2005, 196: 41–46. [[Medline](#)] [[CrossRef](#)]
- 8) Ma J, Zhao L, Nowak TS Jr: Selective, reversible occlusion of the middle cerebral artery in rats by an intraluminal approach. Optimized filament design and methodology. *J Neurosci Methods*, 2006, 156: 76–83. [[Medline](#)] [[CrossRef](#)]
- 9) Heo M, Kim E: Beneficial effects of antecedent exercise training on limb motor function and calpain expression in a rat model of stroke. *J Phys Ther Sci*, 2013, 25: 943–946. [[Medline](#)] [[CrossRef](#)]
- 10) Kim G, Kim E: The effects of antecedent exercise on motor function recovery and brain-derived neurotrophic factor expression after focal cerebral ischemia in rats. *J Phys Ther Sci*, 2013, 25: 553–556. [[Medline](#)]
- 11) Lim SH, Lee JS, Lee JI, et al.: The quantitative assessment of functional impairment and its correlation to infarct volume in rats with transient middle cerebral artery occlusion. *Brain Res*, 2008, 1230: 303–309. [[Medline](#)] [[CrossRef](#)]
- 12) Isayama K, Pitts LH, Nishimura MC: Evaluation of 2,3,5-triphenyltetrazolium chloride staining to delineate rat brain infarcts. *Stroke*, 1991, 22: 1394–1398. [[Medline](#)] [[CrossRef](#)]
- 13) Sughrue ME, Mocco J, Komotar RJ, et al.: An improved test of neurological dysfunction following transient focal cerebral ischemia in rats. *J Neurosci Methods*, 2006, 151: 83–89. [[Medline](#)] [[CrossRef](#)]
- 14) Komotar RJ, Kim GH, Sughrue ME, et al.: Neurologic assessment of somatosensory dysfunction following an experimental rodent model of cerebral ischemia. *Nat Protoc*, 2007, 2: 2345–2347. [[Medline](#)] [[CrossRef](#)]
- 15) Rahman RM, Nair SM, Helps SC, et al.: (-)-Epigallocatechin gallate as an intervention for the acute treatment of cerebral ischemia. *Neurosci Lett*, 2005, 382: 227–230. [[Medline](#)] [[CrossRef](#)]
- 16) Prasad NR, Menon VP, Vasudev V, et al.: Radioprotective effect of sesamol on gamma-radiation induced DNA damage, lipid peroxidation and antioxidants levels in cultured human lymphocytes. *Toxicology*, 2005, 209: 225–235. [[Medline](#)] [[CrossRef](#)]
- 17) Kapadia GJ, Azuine MA, Tokuda H, et al.: Chemopreventive effect of resveratrol, sesamol, sesame oil and sunflower oil in the Epstein-Barr virus early antigen activation assay and the mouse skin two-stage carcinogenesis. *Pharmacol Res*, 2002, 45: 499–505. [[Medline](#)] [[CrossRef](#)]
- 18) Suja KP, Jayalekshmy A, Arumughan C: Free radical scavenging behavior of antioxidant compounds of sesame (*Sesamum indicum* L.) in DPPH(*) system. *J Agric Food Chem*, 2004, 52: 912–915. [[Medline](#)] [[CrossRef](#)]
- 19) Gladstone DJ, Black SE, Hakim AM, Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery: Toward wisdom from failure: lessons from neuroprotective stroke trials and new therapeutic directions. *Stroke*, 2002, 33: 2123–2136. [[Medline](#)] [[CrossRef](#)]