Ginger Pharmacopuncture Improves Cognitive Impairment and Oxidative Stress Following Cerebral Ischemia

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Available online Sep 27, 2012

Received: Jun 22, 2012
Accepted: Aug 30, 2012

KEYWORDS
acupuncture; ginger; oxidative stress; pharmacopuncture; stroke

Abstract
Recent findings have demonstrated that acupuncture and ginger can each improve memory impairment following cerebral ischemia. We hypothesized that ginger pharmacopuncture, a combination of these two treatments, could increase the beneficial effects. Due to the limitation of supporting evidence, we aimed to determine whether ginger pharmacopuncture could improve cognitive function and oxidative stress following cerebral ischemia. Male Wistar rats were induced by right middle cerebral artery occlusion (Rt. MCAO) and subjected to either acupuncture or ginger pharmacopuncture once daily over a period of 14 days after Rt. MCAO. Cognitive function was determined every 7 days, using escape latency and retention time as indices, and the oxidative stress status of the rats was determined at the end of the study. Rats subjected either to acupuncture or to ginger pharmacopuncture at GV20 demonstrated enhanced spatial memory, and the activities of catalase and glutathione peroxidase in both cerebral cortex and hippocampus were improved. Elevation of superoxide dismutase activity was observed only in the hippocampus. Cognitive enhancement was observed sooner with ginger pharmacopuncture than with acupuncture. The cognitive enhancing effect of acupuncture and...
1. Introduction

Stroke is the second most common cause of cognitive impairment and dementia. The prevalence of cognitive impairment following cerebral ischemia is approximate 36.7% [1]. The most common types of cognitive deficits resulting from stroke are disturbances of attention, language syntax, delayed recall and executive dysfunction affecting the ability to analyze, interpret, plan, organize, and execute complex information [2–5]. Hence, this disorder is regarded as a very important health problem. According to Traditional Chinese Medicine, ischemic stroke is regarded as Yin pattern syndrome [6]. Therefore, the goal for treatment is to improve Yin–Yang balance.

Acupuncture has long been used for treating numerous ailments, including stroke and memory deficit. It has been established that acupuncture stimulation of Baihui (GV20) can relieve some of the consequences of stroke, such as headache, anxiety, and dizziness [7–9]. More recently, pharmacopuncture, or herbal acupuncture (an integration of acupuncture and herbal therapies), has been introduced to optimize the benefits of acupuncture and herbal medicine. This type of therapy involves injecting minute quantities of herbs, medicines, self-blood, oxygen, and allergens [10] into acupuncture points.

Ginger (Zingiber officinale), a plant in the Zingiberaceae family, is a culinary spice that has been used as an important herb in Traditional Chinese Medicine for many centuries. Ginger is regarded as a Yang herb, which can decrease Yin and nourish the body. It also possesses antioxidant [11–13], anti-motion sickness [14], and anti-inflammatory activity [15], and improves memory [16] and blood circulation [17]. Moreover, previous work in our laboratory has shown that ginger extract at a dose of 200 mg/kg BW can improve memory deficit in an animal model of ischemic stroke [18].

Since acupuncture at GV20 and ginger extract individually have many health benefits, we hypothesized that the combination of these treatments might further ameliorate brain damage and memory impairment in stroke condition. Since oxidative stress has been reported to play a crucial role in the pathophysiology of ischemic stroke, this study aimed to determine the effect of ginger pharmacopuncture on brain damage in the cerebral cortex and hippocampus and on oxidative stress markers in this area.

2. Materials and methods

2.1. Plant material and preparation of the extract

Z officinale rhizomes were dried for 7 days in the shade at room temperature. These were then ground and the powder was stored in cloth bags at 5 °C until the aqueous extract was prepared. For this purpose, the dried plant material (25 g) was stirred in 250 mL of distilled water for 15 min at 95 °C followed by rapid filtration, first by a crude cellulose filter and then using Whatman #1 filter paper. The average w/w yield was 9.78%.

2.2. Animals

Male Wistar rats weighing 300–350 g were obtained from the National Laboratory Animal Center (Salaya, Nakorn Pathom) and housed in groups of five per cage in standard metal cages at 22 ± 2°C on a 12:12 hour light–dark cycle. All animals were given free access to food and water ad libitum. Experiments were designed to minimize animal suffering, and the experimental protocols were approved by the Institutional Animal Care and Unit Committee, Khon Kaen University, Thailand (Record No. AEKKU 07/2554).

2.3. Induction of focal cerebral ischemia

All experimental rats were fasted for 12 hours but they were allowed free access to water before surgery. Anesthesia was induced by means of intraperitoneal injection of thiopental sodium at a dose of 50 mg/kg body weight. Focal cerebral ischemia was induced as described by Longa et al [19]. Briefly, the right common carotid artery and the right external carotid artery were exposed through a ventral midline neck incision and were ligated proximally. A silicone-coated nylon monofilament (4–0) suture (USS DG; Tyco Healthcare Group Lp, CT, USA), the tip of which was rounded by heating near a flame, was inserted through an arteriotomy in the common carotid artery just below the carotid bifurcation, and advanced into the internal carotid artery approximately 17–18 mm distal to the carotid bifurcation, until a mild resistance was felt. Occlusion of the origins of the anterior cerebral artery, the middle cerebral artery, and the posterior communicating artery was thereby achieved. The wound was sutured, and the rats were returned to their cages with free access to food and water. The incision sites were infiltrated with 10% Povidone–Iodine Solution for antiseptic postoperative care.

2.4. Determination of cognitive function

The water maze consisted of a metal pool (170 cm in diameter × 58 cm deep) filled with tap water (25 °C at 40 cm deep) and divided into four quadrants (NE, NW, SE, and SW) by two imaginary lines crossing the center of the pool. In the center of one quadrant was a removable escape platform. This was below the water level and was covered with a nontoxic milk powder. For each animal, the invisible platform remained at its location at the center of one quadrant throughout training. Rats were tested on their ability to remember the location of the platform in relation to various environmental cues. Each rat was gently placed in the water facing the wall of the pool from one of the four starting points (N, E, S, or W) along the perimeter of the pool, and the animal was allowed to swim until it found and climbed onto the platform. During the training sessions, the rat was gently placed on the platform by the experimenter when it could not reach the platform within 60 seconds. In either case, the rat was left on the platform for 15 seconds before removal from the pool. The time for the animals to climb on the hidden platform was recorded as escape latency. Retention memory was determined the next day. During this
exercise, the platform was removed and the animals were placed into the water maze for 60 seconds. The time taken for an animal to swim to the previous location of the platform was recorded. If the spatial memory of the rat for the trained platform location is accurate, the rat will swim to the previous platform location and search around the exact location. Therefore, the more accurate the animal’s spatial memory is, the greater the number of times the rat will swim across the previous location of the platform. In each trial, the animal was quickly dried with a towel before being returned to the cage [20]. All Morris water maze tests were carried out within 30 minutes of oral administration of the test substances.

2.5. Biochemical assay

Rats were perfused with cold saline solution to get rid of the blood from the brain tissue, then rapidly removed and stored at -80 °C until used.

The lipid peroxidation product, malondialdehyde: malondialdehyde (MDA) was used as an indicator of oxidation. MDA levels were determined via the thiobarbituric acid reaction in tissue homogenate, according to the method of Okawa and colleagues [21]. The colored end product was read at 540 nm. Results were expressed as nmoles MDA/mg protein.

Glutathione Peroxidase (GSH-Px) level: Glutathione peroxidase activity was measured using a spectrophotometric method [22]. This activity was determined using hydrogen peroxide (H$_2$O$_2$) as the substrate and glutathione reductase and nicotinamide adenine dinucleotide phosphate as enzymatic and non-enzymatic indicators, respectively.

Catalase activity determination: Catalase (CAT) activity in the supernatant was measured by recording the rate of decrease in H$_2$O$_2$ absorbance at 240 nm [23]. The activity of CAT was expressed as Units(U)/mg protein.

Superoxide dismutase activity determination: Superoxide dismutase (SOD) activity was measured in the supernatant [24]. SOD activity was expressed in terms of U mg$^{-1}$ protein, where one U was defined as the amount of enzyme that inhibited the optical density at 560 nm of chromogen production by 50% under the above assay conditions.

2.6. Experimental protocol

The animals were induced by middle cerebral artery occlusion (MCAO) and then divided into three groups. Each group consisted of five animals. The needle was twisted at a speed of twice a second for a minute at each point.

Group I: Sham acupuncture plus MCAO group. A non-acupoint, located 5 mm next to Baihui (GV20), was used as the target for needling treatment.

Group II: Acupuncture plus MCAO group. Baihui (GV 20) was used as the target for needling treatment.

Group III: Ginger pharmacopuncture plus MCAO group. A dose of 0.1 mL/kg BW ginger extract was injected into the Baihui (GV 20) point.

All animals received the treatment once daily for a period of 14 days after the occlusion of the right middle cerebral artery. Spatial memory was assessed at 1, 7, and 14 days after MCAO. In order to determine the effect of ginger pharmacopuncture on the alteration of MDA level and the activities of antioxidant enzymes, the animals were divided into groups, as described previously. The brains of all the rats were isolated and prepared for the brain homogenate at the end of the experiment. The level of MDA and the activities of antioxidant enzymes in the brain homogenate were estimated.

2.7. Statistical analysis

All data were expressed as mean ± standard error of mean (SEM) value. Statistical analysis was carried out using SPSS (v. 12.0 for Windows). The statistical significance of the data was determined using one-way analysis of variance (ANOVA). Post-hoc least significance test (LSD) paired comparisons were further made in order to recognize deviant groups. The statistical difference was regarded at p value < 0.05.

3. Results

3.1. Effect of ginger pharmacopuncture on cognitive function

The effects of acupuncture and ginger pharmacopuncture on escape latency and retention time in the Morris water maze test are shown in Figs. 1 and 2. It was found that, at 7 days after Rt. MCAO, the rats subjected to ginger pharmacopuncture exhibited significantly decreased escape latency (p < 0.05), compared with the MCAO + sham acupuncture group. However, at 14 days after MCAO, both rats subjected to acupuncture and rats subjected to ginger pharmacopuncture exhibited significantly decreased escape latency but enhanced retention time (p < 0.05), compared with the MCAO + sham acupuncture group.

3.2. Effect of ginger pharmacopuncture on oxidative stress markers

Based on the crucial role of oxidative stress on the pathophysiology of cerebral ischemic stroke, this study also determined the effect of ginger pharmacopuncture on oxidative stress markers using the level of MDA and activities of various scavenger enzymes, including CAT, glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) as indices.

Figs. 3–6 show that rats subjected to acupuncture and ginger pharmacopuncture had a significantly decreased MDA level both in the cerebral cortex and hippocampus (p < 0.05 all), compared with the MCAO + sham acupuncture group. In addition, it was found that both acupuncture and ginger pharmacopuncture induced significant elevation

![Figure 1](image-url)
of CAT and glutathione peroxidase activities in the cerebral cortex and hippocampus ($p < 0.05$ all), compared with MCAO + sham. However, both acupuncture and ginger pharmacopuncture produced a significant elevation of SOD activity only in the hippocampus ($p < 0.05$ all), compared with MCAO + sham.

4. Discussion

The present study has clearly demonstrated that both acupuncture and ginger pharmacopuncture can improve cognitive deficit and oxidative stress damage following stroke. In addition, ginger pharmacopuncture can enhance memory function more rapidly than acupuncture.

Ischemic stroke, the most common type of stroke, occurs as the result of a thrombotic or embolic occlusion of a major cerebral artery, especially the middle cerebral artery. Several experimental focal ischemia models have been proposed to mimic the pathophysiology of ischemic stroke in recent decades\[25\]. Among these diverse models, intraluminal suture MCAO in rats is the most frequently used model because it is both less invasive than other models and easier to perform in a controlled manner. As the MCAO model can provide higher ischemic lesion growth size within 24 hours, it may be beneficial in studies of neuroprotective strategies. Hence, we employed the MCAO injury model to test the efficacy of ginger pharmacopuncture.

Accumulative lines of evidence have revealed that oxidative stress plays a pivotal role in the neuronal injury that occurs during brain ischemia. It has been found that free radicals, such as the superoxide anion, hydroxyl radical, and $\text{H}_2\text{O}_2$ are elevated during stroke, whereas constituents of the endogenous antioxidant system, including the antioxidant enzymes, SOD, GSH-Px, and CAT and the antioxidants, glutathione (GSH), vitamin (vit) C and vit E ($\alpha$-tocopherol) are decreased\[26\].

Previous investigations have shown that acupuncture at GV20 can improve spatial memory impairment following cerebral ischemia at the cellular level\[27,28\]. In addition, electroacupuncture at Baihui (GV20), Yanglingquan (GB34), Taichong (LR3), Zusanli (ST36) and Xuehai (SP10) can decrease reactive oxygen species generation and the MDA level in accompaniment with the increase in SOD\[29\]. Our findings correlate well with these previous results, in that decreased MDA level and increased SOD, CAT, and GSH-Px activity was accompanied by improvement in memory impairment. In addition, ginger pharmacopuncture was shown to improve memory impairment more rapidly than regular acupuncture, although the pattern and magnitude of the changes in oxidative stress were similar for both treatments. This suggests that another mechanism beyond oxidative damage might be involved in causing memory impairment. Although determination of this mechanism is beyond the scope of the present study, we suggest that ginger may affect neurotransmitters such as acetylcholine, norepinephrine (NE), dopamine (DA), and serotonin, which...
play a crucial role in learning and memory [30–32]. The precise mechanism underlying this effect requires further investigation, however.

In conclusion, the current study clearly demonstrates that ginger pharmacopuncture at GV20 can improve memory impairment following cerebral ischemia more rapidly than acupuncture. One probable mechanism underlying this effect is improved oxidative stress. However, other mechanisms may also contribute to the cognitive enhancing effect of ginger pharmacopuncture, and this requires further exploration.

Acknowledgments

This study was supported by the Integrative Complimentary Alternative Medicine Research and Development Group, Khon Kaen University, Khon Kaen, Thailand.

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