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Prevention and Repair of Cerebral Ischemia–Reperfusion Injury by Chinese Herbal Medicine, Shengmai San, in Rats

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The protective activity of Shengmai San, a traditional Chinese herbal medicine, was studied in cerebral ischemia–reperfusion injury in rats. Shengmai San consists of three herbal components, *Panax Ginseng*, *Ophiopogon Japonicus* and *Schisandra Chinensis* and is routinely being used for treating coronary heart disease.

When Shengmai San was injected directly into rat duodenum 2 h before cerebral ischemia by bilateral carotid artery occlusion, thiobarbituric acid reactive substance (TBARS) formation during reperfusion following ischemia was almost completely suppressed in the brain. The loss of glutathione peroxidase activity after the ischemia–reperfusion was also effectively prevented by the Shengmai San pre-administration whereas the activity was considerably decreased in the damaged brain.

It was found that Shengmai San also effectively suppressed the TBARS formation even when it was administered after 45 min reperfusion following ischemia, indicating that Shengmai San improves the oxidative damage already established in the brain. Likewise, the decrease of glutathione peroxidase activity was minimized in the damaged brain by the post-administration of Shengmai San.

On the other hand, none of the Shengmai San components were active in protecting the ischemia–reperfusion brain damage when they were independently administered.

These experiments suggest the potential of Shengmai San in both preventive and therapeutic usages for cerebral ischemia–reperfusion injury.

Keywords: Shengmai San, cerebral ischemia–reperfusion, oxygen stress, traditional Chinese herb medicine, glutathione peroxidase, thiobarbituric acid reactive substance (TBARS)

Abbreviations: NADPH, β-nicotinamide adenine dinucleotide phosphate reduced form; GSH, glutathione reduced form; GPX, glutathione peroxidase; EDTA, ethylenediamine tetraacetic acid

INTRODUCTION

Reactive oxygen (ROS) and nitrogen species have been implicated in several physiological and pathological phenomena.[1,2] The brain is considered to be a prime target of ROS mediated damage because of its high lipid content, high rate of

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oxidative metabolism, and relatively low level of free radical eliminating enzymes. Indeed, there is considerable evidence for the involvement of free radicals and lipid peroxidation in the pathophysiology of brain ischemia–reperfusion.[3–8] Lipoperoxy radicals thus produced can initiate and propagate oxidative chain reactions to damage further the cellular components such as protein and DNA,[9] to decrease physiological antioxidant levels such as GSH,[10] and eventually lead to brain pathological conditions. Several attempts have been reported to prevent oxidative stress in the brain using antioxidant molecules or inhibitors of ROS production such as polyethylene glycol conjugated superoxide dismutase and catalase,[11] allopurinol,[12] and α-lipoic acid,[10] but so far only limited success was obtained.

Traditional Chinese herbal medicines have been used for treating a variety of physiological disorders. Although the precise mechanism of their action is not yet completely understood, manipulation of autonomic nervous function may be one of their effects. Immune regulating function has also been suggested[13] indicating that ROS scavenging activity is involved in their function. Therefore, it is of interest to study the use of Chinese herbal medicine as a modulator of oxidative stress in the brain.

We studied here the effect of Shengmai San on oxidative brain damage after ischemia–reperfusion in rats. Shengmai San, consisting of three herbs, *Panax Ginseng*, *Ophiopogon Japonicus* and *Schisandra Chinensis*, has been clinically used for the treatment of coronary heart disease.[14,15]

As a biochemical marker of oxidative damage, GPX activity was measured besides TBARS to evaluate peroxide scavenging potential, although the brain level of free radical eliminating enzymes is known to be relatively low. The results obtained here show that Shengmai San has an ability to prevent brain oxidative damage during ischemia–reperfusion and moreover, that Shengmai San administered after long reperfusion following ischemia is still considerably effective for improving oxidative damage in the brain.

MATERIALS AND METHODS

Chemical and Herb Materials

Constituent herbs of Shengmai San formula (*Panax Ginseng* C.A. Meyer, product of Aize, Japan; *Ophiopogon Japonicus* Ker-Gawler, product of Sichuan Sheng, P.R. China; *Schisandra Chinensis* Billon, product of Jilin Sheng, P.R. China) were obtained from Magiya pharmacy Co., in Niigata, Japan. 2-thiobarbituric acid (TBA) and GSH were purchased from Wako Co., Ltd. Disodium EDTA from Kanto Chemical Co., Ltd. Sodium dodecyl sulfate (SDS) from Nakarai Co., Ltd. β-NADPH and GSH reductase were from Sigma Co., Ltd. All other chemicals were of reagent grade and used without further purification.

Preparation of Shengmai San

Shengmai San was prepared according to the traditional prescription. Briefly, a total of 5 g of herbal composite consisting of 2 g *Panax Ginseng*, 2 g *Ophiopogon Japonicus* and 1 g *Schisandra Chinensis* was soaked in 50 ml distilled water for 1 h, then boiled gently for 30 min. The supernatant was separated by decantation, then filtered using delipidated gauze, and the filtrate was stored in a refrigerator until use.

Animal Treatment

Male Wistar rats (6 weeks old and 182–196 g body weight) were purchased from SLC Inc., Japan. They were allowed free access to pelletted diet and water.

Anesthesia was induced by diethyl ether and maintained by pentobarbital. For examining the preventive effect of Shengmai San on cerebral ischemia–reperfusion injury, Shengmai San extract at a dosage of 5 g crude herb mixture per kg body weight was administered directly into the lumen of duodenum exposed by abdomen incision 2 h before ischemia operation. Cerebral ischemia was produced by the occlusion of both

right and left common carotid arteries exposed through a middle skin incision using aneurysm clips for 85 min duration. At the end of the ischemic period, carotid arteries were declamped to allow blood reperfusion. All rats were sacrificed under anesthesia with pentobarbital after reperfusion treatment, then the brain was taken out for biochemical analysis.

For examining the later effects of Shengmai San, the extract was injected after 45 min reperfusion following 85 min ischemia, then the reperfusion was continued for another 2 h before the animals were sacrificed.

For each experiment, biochemical measurements were carried out for three groups of rats: 1. Normal control: rats not given any treatment. 2. Saline control: rats administered normal saline before/after bilateral carotid artery occlusion followed by reperfusion. 3. Shengmai San treated: rats treated with Shengmai San before/after cerebral ischemia–reperfusion as mentioned above.

Tissue Homogenates

After ischemia–reperfusion treatment, the whole brain was removed, weighed, and chilled in ice-cold saline. After washing with 0.85% NaCl, the tissues were suspended in an aliquot of cold 1.15% (w/v) KCl (9 ml per 1 g wet tissue), then homogenized at 0°C using a glass homogenizer.

Analytical Procedures

TBARS formation was determined by the method reported previously.[16] Briefly, an aliquot of brain homogenate was mixed with 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA, then the volume was adjusted to 4.0 ml with distilled water. After boiling at 95°C for 60 min, the reaction solution was extracted with 1.0 ml of distilled water and 5.0 ml of *n*-butanol and pyridine (15 : 1 v/v). The absorbance at 532 nm of the organic layer was determined after centrifugation.

GPX activity was determined according to the method of Albrecht.[17] Briefly, an aliquot of brain homogenate (4 mg wet tissue) in 0.05 M phosphate buffer containing 1.15% (w/v) KCl was mixed in a quartz cuvette with 935 µl of the coupling solution prepared by dissolving 33.6 mg disodium EDTA, 6.5 mg NaN_3, 30.7 mg of GSH, 16.7 mg NADPH and 100 units of GSH reductase in 100 ml of 50 mM Tris-HCl (pH 7.6). Kinetic decay of NADPH fluorescence (Ex. 355 nm/Em. 465 nm) was measured after the addition of 25 µl of 1 mM H_2O_2 as substrate using a Hitachi model 650-60 fluorescence spectrophotometer.

Triplicate determinations were carried out for each brain homogenate and the data are given as an average ± SD of 6 rats. Data were evaluated by the Student's *t*-test and a P value < 0.05 was accepted as statistically significant.

RESULTS

The protective effect of Shengmai San on oxidative stress induced cerebral damage was studied. Shengmai San was injected directly into the lumen of duodenum using a syringe 2 h before ischemic operation, then TBARS formation and GPX activity were determined in the brain after 45 min reperfusion following 85 min ischemia. TBARS in whole brain homogenate was considerably increased in the brain damaged after ischemia–reperfusion compared to untreated normal brain (187.2% of normal control). However, in the Shengmai San treated group, no significant increase of TBARS was determined as shown in Figure 1. The brain TBARS level of the Shengmai San administered group was almost the same level as the normal rat group indicating TBARS formation was completely inhibited by the Shengmai San pre-administration.

The effective protection of brain oxidative damage by Shengmai San was further demonstrated by determining GPX activity in the whole brain homogenate. GPX activity was found to decrease markedly in the brain after

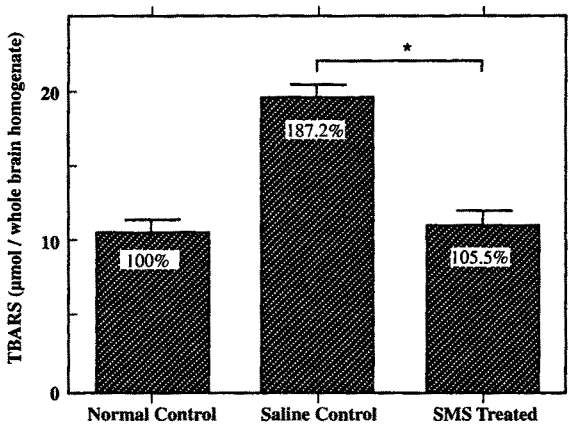

FIGURE 1 Preventive effect of Shengmai San on lipid peroxidation in rat brain after ischemia followed by reperfusion. Shengmai San was administered into rat duodenum 2 h before the brain was subjected to ischemia condition by carotid arteries occlusion. After 45 min reperfusion following 85 min ischemia, TBARS was determined for the whole brain homogenate. Triplicate determinations were done for one brain homogenate. The data are given per whole brain homogenate because of the water content change in the damaged brain. Values are given as mean of 6 rats with SD. $^*p < 0.05$.

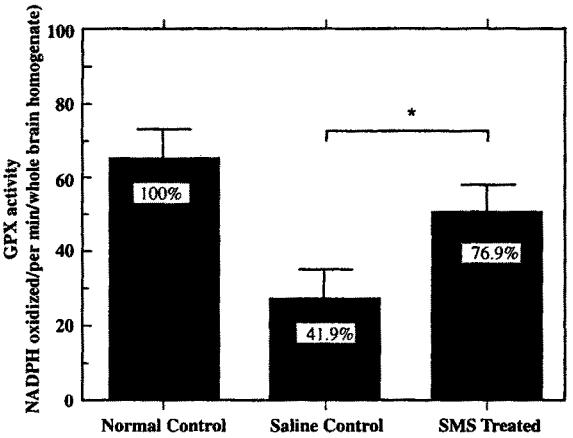

FIGURE 2 Preventive effect of Shengmai San on GPX activity in the rat brain after ischemia followed by reperfusion. Experimental conditions and the data processing are same as in Figure 1.

ischemia–reperfusion but the activity loss was effectively prevented by Shengmai San pre-administration although the protecting efficiency against GPX was somewhat lower than against TBARS formation. Approximately 77% of the normal activity was retained after ischemia–reperfusion injury whereas only 42% of the activity was preserved in the saline control (Figure 2). This corresponds to approximately 60% inhibition of the ischemia–reperfusion induced GPX activity loss.

None of the components of Shengmai San formula were able to prevent the ischemia–reperfusion damage when they were administered independently. For example, TBARS formation was slightly inhibited by *Schisandra* but not at all by *Panax* and *Ophiopogon*. The GPX preserving activities of both *Panax* and *Schisandra* were approximately 20% of the complete Shengmai San formula, and that of *Ophiopogon* was only 10% (data not shown).

In order to know whether Shengmai San is also effective to decrease the oxidative damage already established in the brain after ischemia–reperfusion, Shengmai San was administered after 45 min reperfusion following ischemia, then the TBARS in the brain was determined after an additional 2 h reperfusion treatment. TBARS level of saline control slightly increased to 199% of normal control with longer reperfusion. Even under this condition, Shengmai San was found to effectively inhibit the TBARS formation in the damaged brain. In the Shengmai San treated animals, the TBARS level was 133% of normal control (Figure 3) indicating approximately 67% of ischemia–reperfusion induced TBARS formation was inhibited even though Shengmai San was administered after reperfusion damage occurred.

The post-administration effectiveness of Shengmai San was further confirmed when GPX activity was measured. It becomes obvious that more critical damage occurs in the brain by the longer reperfusion following ischemia such that only 22% of normal GPX activity was preserved after an additional 2 h reperfusion (cf. 42% after 45 min reperfusion). In the Shengmai San treated group, however, the enzyme level was still maintained at 64% of normal control (Figure 4)

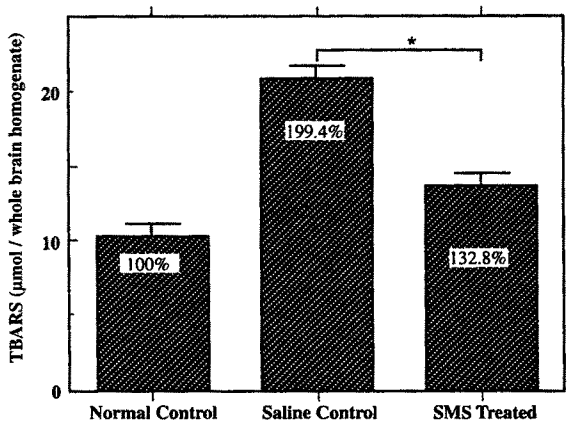

FIGURE 3 Effect of Shengmai San administered after reperfusion following ischemia on TBARS in the rat brain. Shengmai San was administered to rats after 45 min reperfusion following 85 min ischemia. TBARS was determined in the brain reperfused additional 2 h. Triplicate determinations were done for one brain homogenate. The data are given per whole brain homogenate because of the water content change in the damaged brain. Values are given as mean of 6 rats with SD. $*p < 0.05$.

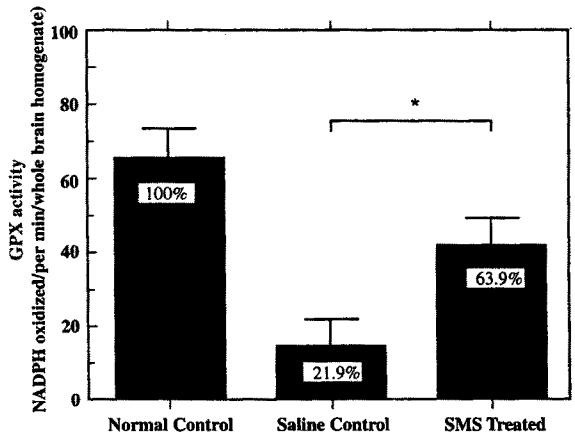

FIGURE 4 Effect of Shengmai San administered after reperfusion following ischemia on GPX activity in the brain. Experimental conditions and the data processing are same as in Figure 3.

corresponding to 54% inhibition of the ischemia–reperfusion induced enzyme loss.

DISCUSSION

The roles of free radicals and their mediated lipid peroxidation have been recognized in the pathophysiology of brain ischemia–reperfusion.[3–9,18] Antioxidant therapy, aimed at reducing the extent of free-radical-mediated tissue damage, thus represents a rational approach for preventing the onset and/or progression of free-radical-related pathogenesis. Therefore, the measurement of antioxidant activity should form an additional basis for drug screening and selection.[19] Chinese herbal medicines should be one of the targets to be studied because they have a complex formula comprising several herbal materials, some of which are known to be potent antioxidants.[20,21] In the present study, we examined the antioxidant potential of traditional Chinese medicine, Shengmai San, in cerebral ischemia–reperfusion injury in rat.

Result obtained here clearly showed that Shengmai San has a strong activity to prevent the brain oxidative damage produced by ischemia–reperfusion. Pre-administration of Shengmai San successfully prevented the critical loss of GPX activity and decreased TBARS formation in the brain after ischemia followed by reperfusion.

Moreover, it was revealed that Shengmai San administered long after the reperfusion following ischemia was still effective both in preventing TBARS accumulation and GPX activity loss in the brain already damaged to considerable extent. The activity of Shengmai San against established oxidative damage was clear when its effects were compared on the brain tissues reperfused for different durations. In the brain reperfused for 165 min following ischemia, the TBARS was elevated to 199% of normal level compared to 187% for 45 min reperfusion. In the Shengmai San administered brains, the TBARS values were 133% and 106% of normal for each reperfusion period, respectively, thus TBARS formation was markedly inhibited in both brains. However, a big difference was observed in the inhibitory action on TBARS in the rats administered with Shengmai San before and after ischemia–reperfusion (94% inhibition vs 67% inhibition).

By contrast, Shengmai San dependent recovery of GPX activity was almost comparable in the

rats administered with Shengmai San before (60% recovery) and after the ischemia–reperfusion (54% recovery), although the GPX activity was more depleted by the longer reperfusion than the shorter one in saline control. Thus GPX activity was more effectively improved than TBARS by the Shengmai San administered after ischemia–reperfusion whereas the prevention effect of Shengmai San was more clearly demonstrated on TBARS than on GPX activity.

There is little doubt that free radical reactions play a major role in initiation and propagation of ischemic injury. However, many other biochemical and physiological processes are also involved in establishing oxidative damage in the brain.[5] Therefore, antioxidant therapy using a single active compound will have inherent limitations, especially for improving the damage already established, whereas certain success has been reported in preventing further development of oxidative damage.[10–12,22] To overcome this limitation, a combination therapy would be promising in that antioxidants are formulated with other biomodulators participating in such processes as repair enzymes induction. Chinese traditional herbal medicines with antioxidant activity are an attractive formula in this sense because they usually consist of several herbal constituents having different physiological functions. The present study indeed suggested that TBARS and GPX are differently controlled by Shengmai San.

Although further study is needed to show functional improvement of the damaged brain, the data in the present study suggest that Shengmai San could be a potential herbal medicine for treating brain oxidative injury mediated by reperfusion following cerebral ischemia.

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