대한중풍학회지 제10권 제1호 (2009년 12월)
The Korean Journal of Joongpoong 2009;10(1):8-19

원 저

The Effect of *Scutellariae Radix* on Ischemia Induced Brain Injury in Rats

Ji-Eun Park, Young-Kyun Kim

Department of Oriental Medicine, Dong Eui University

Scutellaria Radix, originated from *Scutellaria baicalensis* Georgi, is one of the most important medicine in traditional Oriental medicine, and possesses anti-bacterial activity and sedative effects, can be applied in the treatment of a range of conditions including diarrhea and hepatitis. It is reported that chronic global ischemia induces neuronal damage in selective, vulnerable regions of the brain, especially the hippocampus and cerebral cortex. In the present study, to investigate the effect of *Scutellaria Radix* extract on cerebral disease, the changes of regional cerebral blood flow and pial arterial diameter on ischemia/reperfusion state was determinated by Laser-Doppler Flowmetry and some parameters concerned with oxidative stress also measured.

When SRe were administered for five days with the concentration of 100 ㎎/㎏, GSH activity significantly increased. But SRe administeration showed no significant change in lipid peroxidation. When the activities of CAT, Cu, Zn-SOD and GSH were measured, CAT and GSH were activated by SRe administration.

When 1 and 3 ㎍/㎖ SRe was applied to the neuronal cell cultures, the quantities of LDH was significantly reduced when compared with cultures treated only with NMDA.

Through this study, it can be concluded that the ischemia/reperfusion induced brain stress may have contributed to cerebral damage in rats, and the present study provides clear evidence for the beneficial effect of SRe on ischemia induced brain injury.

Key Words : *Scutellaria Radix*, ischemia, reperfusion, SRe

Introduction

With an increasing elderly population, various aging-related diseases such as hypertension, arteriosclerosis and different forms of dementia are also increasing. According to clinical observation, the patients who are characterized by intellectual declines often suffer from global ischemia, global ischemia/hypoxia, or cerebral hemorrhage-induced vascular disorder[1]. It is reported that chronic global ischemia induces neuronal damage in selective, vulnerable regions of the brain, especially the hippocampus and cerebral cortex[2]. Further, abnormal levels in the brain of a series of metabolites such as acetylcholinesterase, glucose, lactate, ATPase, cytochrome oxidase, NOS and free radicals resulted from the neuronal damage and these chronically impaired neuronal productions can lead to deficits in learning and memory[3].

After cancer, stroke is the most frequent cause of death in South Korea, more frequent than heart disease[4]. Despite Korea's rapid industrialization and modernization, mortality from stroke has not decreased until recently. The high incidence of stroke is due, at least in part, to the aging of the population, but there is also evidence that stroke prevention is inadequate

교신저자 : 김영균
주소 : 부산광역시 부산진구 양정2동 산 45-1
동의대학교 부속 한방병원 한방 2내과
전화 : 051-850-8620 팩스 : 051-867-5162 E-mail : ykkim@demc.or.kr

in South Korea.

Traditional Oriental medicine has been used to heal many diseases for thousands of years and is now well known as natural medicine throughout the world.

Scutellaria Radix, originated from *Scutellaria baicalensis Georgi*, is one of the most important medicine in traditional Oriental medicine. It possesses anti-bacterial activity and sedative effects[5], and finds application in the treatment of a range of conditions including dizzness, diarrhea and hepatitis[6]. Wogonin, the major chemical constituent of this herb, is a flavone derivative containing a phenylbenzopyrone nucleus. Flavonoids can be isolated from a wide variety of fruits, vegetables, nuts, and flowers, as well as tea, which are important components of the human diet[7]. The biological and pharmacological properties of flavonoids are broad and include anti-viral and anti-inflammatory actions[8], the reduction of neuronal oxidative metabolism[9], estrogenic effects[10], and the inhibition of enzymes including protein kinase C and tyrosine kinase[11].

In the present study, to investigate the effect of *Scutellaria Radix* extract(SRe) on cerebral disease, the changes of regional cerebral blood flow(rCBF) and pial arterial diameter(PAD) on ischemia/reperfusion state was determinated by Laser-Doppler Flowmetry (LDF) and some parameters cincerned with oxidative stress also measured.

Materials and Methods

1. *Scutellariae Radix* extract (SRe) preparation

SRe was purchased in the special herb market (Omni Herb, Korea) and carefully selected good samples. To fractionate the aqueous extract, 100 g of the dried herbs consist SRe was boiled with 1500 ㎖ of pure water at 100℃ for 2 hours. After filteration, the filtrate was evaporated under reduced pressure and then freeze-dried to obtain the aqueous extract. And the total crude extractive powder was 11.3 g. The extract stored in deep freezer when unused, and freshly diluted for experiment. Rats were fed orally with the dose of 100㎎/㎏/day for five days when needed.

2. Animals and chemicals

Adult male Sprague-Dawley rats at the body weight of 200±20 g were obtained commercially (Daehan experimental animal, Korea) and used. All animals were housed under standard conditions of lights and controlled room temperature, and received food and water ad libitum. All chemicals were purchased from Sigma Chemical Co.(Sigma-Aldrich Korea, Korea).

3. Middle cerebral artery occlusion surgery procedure

Middle cerebral artery occlusion(MCAO) was carried out according to the method of Zea-Longa et al.. Male Sprague-Dawley rats were anaesthetised and the left middle cerebral artery(MCA) was occluded by an intraluminal filament at the origin from the Circle of Willis. Briefly, the left common carotid artery(CCA) was exposed through a midline incision. The internal carotid artery(ICA) was then isolated and its branch, the pterygopalatine artery, was ligated close to its origin. Then a 2 ㎝ length of 5-0 rounded dermalon suture with a slightly larger tip was carefully advanced from the external carotid artery through the CCA and then up to the ICA for a distance of 11±0.5 ㎜. The production of ischemia was confirmed by rCBF measurement using a LDF. Following 120 minutes of MCAO, cerebral blood reperfused.

4. Measurement of rCBF in rats

Rats were anesthetized with urethane(750 ㎎/㎏, i.p.) and placed on a stereotaxic frame. Tracheotomized and entubated, and body temperature was maintained at 37℃ with a servo-controlled heating lamp and a rectal thermistor. One femoral artery was used for the

monitoring of blood pressure with PE-50 polyethylene catheters and were recorded on a polygraph.

The skull was exposed and a hole 5 ㎜ in diameter was drilled in the left side at a site 5 ㎜ lateral and 2 ㎜ posterior to the bregma. A laser-Doppler flowmeter(Transonic Instrument, USA) with a 0.8 ㎜ needle probe was used to determine changes in rCBF. The probe tip was positioned above the surface of the intact dura and fixed to a support attached to the skull.

5. Pial arterial diameter measurement

Each rat was anesthetized, then mechanically ventilated through a tracheostomy tube by means of a ventilator(60/min; KN-56, Natsume Seisakusho Co. Ltd. Tokyo, Japan) using room air supplemented with oxygen. The femoral artery was cannulated both for the continuous measurement of arterial blood pressure and to provide blood samples for the determination of arterial blood gas tensions, pH, glucose, and serum electrolytes. The femoral vein was cannulated for administration of fluid and drugs. Body temperature was maintained at between 37℃ and 38℃ by means of a heating pad. A closed cranial window was used for observation of the pial microcirculation, the head being fixed in the sphinx position. The scalp was retracted, a 3×2 ㎜-diameter hole made in the bone over the right parietal cortex, and the dura opened carefully. A polypropylene ring with a fitted glass coverslip was placed over the hole and secured with dental acrylic, and the space under the window was filled with artificial cerebrospinal fluid. The temperature within the window, which was monitored using a thermistor(Model 6510; Mallincrodt Medical; St. Louis, MO, USA), was maintained at 37-38℃. The pial views obtained in these experiments were stored on videotape for later playback and analysis. In each rat, the diameters of three pial arterioles(baseline diameters, 26 to 82 ㎛) were measured using a video-micrometer(Olympus Flovel videomicrometer, Model VM-20; Flovel, Tokyo, Japan) on a television monitor that received signals from a microscope(Model SHZ-10; Olympus, Tokyo, Japan).

6. Surgical procedure of bilateral clamping of the common carotid arteries(BCCA)

Rats were subjected to 30 min of bilateral clamping of the common carotid arteries(BCCA) by wrapping thread around the artery to occlude the flow of blood. The BCCA procedure was carried out under ketamine (50 ㎎/㎖, i.p.) + xylazinum(20 ㎎/㎖, i.p.) anesthesia. The cessation of carotid blood flow was controlled visually. After the occlusion period the threads were removed and the surrounding skin was sutured. Sham-operated animals had their carotid artery exposed for the same period of time without clamping. During anesthesia and surgery, the mice were breathing spontaneously and the rectal temperature was kept at 37℃ by a heating pad.

7. Transfer latency and frequency in elevated plus-maze

Five days after the BCCA surgery, transfer latency and frequency in the plus-maze were measured to evaluate the motor activity. The plus-maze consisted of two opposite open arms, 50×10 ㎝, crossed with two enclosed arms, of the same dimensions with 40 ㎝ high walls. The arms were connected with a central square(10×10 ㎝) to give the apparatus a plus sign appearance. The maze was kept in a dimly lit room elevated 50 ㎝ above floor level. A rat was individually placed on the end of one of the open arms, facing away from the center, and the time taken by the animal to enter one of the closed arms(transfer latency, TL) and frequency of entries during 300 sec were recorded with the help of a stop watch.

8. Preparation of brain homogenates

Five days after the BCCA surgery, the brains were

dissected while still frozen as previously described [12]. The striatum were isolated and weighted. The tissues were homogenized with an ultrasonic dismembrator for 12 rapid pulses in ice-cold 0.1 M phosphate-buffered saline(PBS, pH 7.4) containing 0.1 mM EDTA. The homogenates were centrifuged at 14,000 ×g for 30 min at 4℃, and the supernatants were collected. Protein concentrations were determined using the Bio-Rad protein assay kit using bovine serum albumin as a standard.

9. Measurement of GSH

Reduced glutathione(GSH) concentration in brain homogenate was measured by a commercial kit supplied by Cayman Chemical Co. This kit utilizes an optimized enzymatic GR recycling method for quantification of GSH18). Briefly, 100 ㎕ of brain homogenate was added to an equal volume of the metaphosphoric acid and then centrifuged at 2,000×g for 2 min to remove protein. Then, 50 ㎕ of 4 M triethanolamine was added for each milliliter of homogenate to increase the pH. For total GSH assay, 50 ㎕ of sample was added to 150 ㎕ of a reaction mixture containing 0.4 M 2-(N-morpholino) ethanesulfonic acid, 0.1 M phosphate(pH 6.0), 2 mM EDTA, 0.24 mM NADPH, 0.1 mM 5,5′-dithiobis-2-nitrobenzoic acid(DTNB), and 0.1 unit GR. The reaction was carried out at 37℃ for 25 min, and then total glutathione was determined by absorbance at 412 nm using GSSG as standard. The amount of reduced GSH was obtained by subtracting GSSG from total glutathione. Each sample was assessed in duplicates, and the levels of GSH were expressed as μmol/g tissue.

10. Measurement of lipid peroxidation

Lipid peroxidation results in a formation of LPO that can therefore be used to indicate the levels of lipid peroxidation in tissue. LPO in brain homogenate was quantified with the Cayman's LPO assay kit. This kit measures hydroperoxides directly utilizing the redox reactions with ferrous ions[13]. Briefly, 100 ㎕ of brain homogenate was added to an equal volume of saturated methanol and 1 ml of cold chloroform. The mixture was mixed thoroughly and then centrifuged at 2000×g for 5 min to extract LPO into chloroform layer. Then, 500 ㎕ of chloroform extract was mixed with 450 ㎕ of chloroform/methanol solvent and 50 ㎕ of freshly prepared chromogen (containing 4.5 mM ferrous sulfate in 0.2 M hydrochloric acid) in glass tube. Absorbance was measured at 500 nm after 5 min incubation. The LPO levels in sample homogenates were calculated with standard curve of LPO. Each homogenate was assessed in duplicates, and LPO was expressed as nmol/g tissue.

11. Measurements of plasma catalase(CAT) activity

The whole blood from the heart of rat was centrifuged at 1500×g for 10 min at 4℃ to separate plasma from erythrocytes, and the plasma was used for some measurement. Catalase activity was measured by the method of Aebi using hydrogen peroxide as substrate. The disappearance of H_2O_2 was followed at 240 nm. Enzyme activity was expressed as k/g protein(k: rate constant ofa first-order reaction) at 25℃.

12. Measurement of plasma Cu, Zn-SOD activity

Cu, Zn-SOD activity was measured according to the method of Misra and Fridovich. This method is based on the ability of Cu, Zn-SOD to inhibit the auto-oxidation of adrenalin to adrenochrome at alkaline pH. Enzyme activity was expressed as U/g protein at 30℃.

13. Measurement of plasma GSH

Glutathione level was assayed using the methods of Sedlak and Lindsay and Fairbanks and Klee. 5,5'-Dithiobis-(2-nitrobenzoic acid)(DTNB) is a disulfide

chromogen and turns to dark yellow by reducing with sulphydryl compounds. The absorbance of reduced chromogen was followed at 412 nm. GSH levels were determined from a standard curve and expressed as ㎎/g protein.

14. Measurement of plasma lipid peroxidation

Lipid peroxidation in hepatic tissue and serum was estimated by measuring the content of malondialdehyde (MDA) according to the method of Uchiyama and Mihara. Samples were homogenized in ice-cold 1.15% KCl(5% wt/vol). A 0.5 ㎖ of homogenate was added to 3 ㎖ of 1% phosphoric acid and 1 ㎖ of 0.6% thiobarbituric acid. The mixture was heated for 45 min on a boiling water bath. After addition of 4 ㎖ of n-butanol, the contents were vigorously vortexed and centrifuged at 200×g for 20min. The absorbance of the upper, organic layer was measured at 535 and 520 nm with diode array spectrophotometer, and was compared to results obtained using freshly prepared malondialdehyde tetraethylacetal standards. MDA values were expressed pmoles per ㎎ protein. Protein was measured by the method of Bradford.

15. Primary neuronal cell culture

Primary neuronal cultures were obtained from the cerebral cortex of fetal rats(17-19 days of gestation) according to the procedures of Taguchi et al.[14]. Briefly, pregnant rats were anesthetized with sodium pentobarbital, and the cerebral cortex of fetal rats were rapidly removed bilaterally and collected in ice-cold Hanks' solution(137 mM NaCl, 5.4 mM KCl, 3.4 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 5.6 mM d-(+)-glucose, 2.4 mM HEPES, pH 7.2). The cerebral cortex was mechanically dissociated with a scalpel and by trituration with a Pasteur pipette. Dissociated cells were filtered through a stainless steel mesh with a pore size of 150 ㎛ and centrifuged at 200×g for 3 min. The supernatant was removed, and cells were resuspended in complete medium(Eagle's MEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 11 mM d-(+)-glucose, 24 mM $NaHCO_3$ and 10 mM HEPES). Finally, single cell suspensions were plated onto 0.1% polyethylene-imine-coated plates at a glass coverslips at a density of $1.8 - 4.8 \times 10^5$ cells/㎖. Cultures were maintained in above-mentioned complete medium(1-8 days after plating) at a Eagle's MEM supplemented with 10% heat-inactivated horse serum, 2 mM l-glutamine, 11 mM d-(+)-glucose, 24 mM $NaHCO_3$ and 10 mM HEPES(9-15 days after plating). Cultures were incubated at 37 ℃ in a humidified 5% CO_2 atmosphere. To prevent proliferation of non-neuronal cells, 10 μM cytosine β-d-arabinofuranoside hydrochloride was added after 6 days of plating. Culture medium was changed every two days.

16. Evaluation of neurotoxicity

Cytotoxicity was quantified by measurement of LDH released into culture medium during drug exposure, with a Cytotoxicity Detection LDH kit according to the manufacturer's instructions with slight modifications. In this colorimetric assay, β-nicotinamide adenine dinucleotide(β-NAD) is reduced to NADH through the conversion of lactate to pyruvate by LDH, and then NADH reduces tetrazolium into formazan dyes in the presence of diaphorase. After treatment of cultures with NMDA or SRe for 24 hrs, 10 ㎕ of culture supernatant was mixed with 90 ㎕ of coloring reagent in a 96-well plate at room temperature. After incubation for 1 h, the reaction was stopped by addition of 100 ㎕ of 1 N HCl, and the absorbance at 570 nm was measured with a microplate reader(Model 550, Bio-Rad Laboratories, Inc.).The background absorbance obtained from the culture medium was subtracted from all values.

17. Data analysis

The data are expressed as the mean±SE. The diffe-

rences between groups were analyzed by Student's t-test. The significance level was set at $p<0.05$.

Results

1. Time course effect of SRe treatment in cerebral ischemia/reperfusion induced changes of rCBF and PAD

Middle cerebral artery occlusion(MCAO) was carried out according to the method of Zea-Longa et al.. Following 120 minutes of MCAO, cerebral blood reperfused. SRe treatment showed no significant change on cerebral hemodynamics of cerebral ischemia/ reperfusion(Fig. 1), and the same on the cerebral blood vessel diameter by increasing PAD(Fig. 2).

2. Transfer latency and frequency in elevated plus-maze

In BCCA operation applied rats, SRe treatment showed an anxiolytic-like effect at 100 ㎎/㎏ for five days. The vehicle treated control group typically avoided spending time on or entering into enclosed arms(Fig. 3). Vehicle treated rats remained for 72.8± 8.6 sec in the open arms, whereas SRe treated rats spent significantly more time in the open arms. But on the moving frequency, SRe treated rats showed no significant change(Fig. 4)

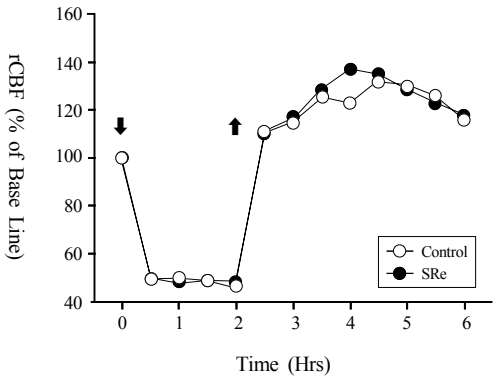

Fig. 1. Time course effect of SRe treatment on the changes in rCBF induced by cerebral ischemia /reperfusion.

↓, Occlusion of middle cerebral artery
↑, reperfusion of blood.

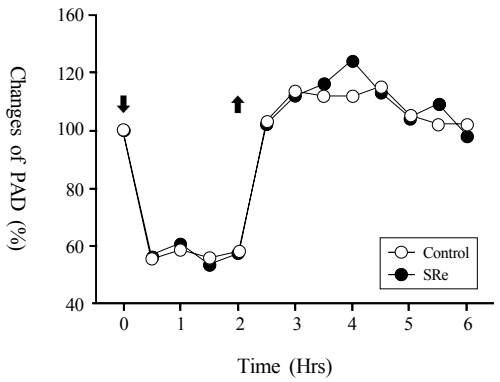

Fig. 2. Time course effect of SRe treatment on the changes in PAD induced by cerebral ischemia/reperfusion.

↓, Occlusion of middle cerebral artery
↑, reperfusion of blood.

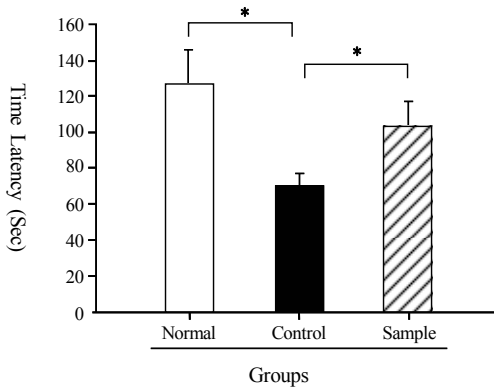

Fig. 3. Effects of SRe on the BCCA surgery on the time spent in open arms of the elevated plus-maze test in rat. Values represent mean±S.E. of six rats per group.

Normal, sham operated and vehicle administered group.
Control, BCCA operated and vehicle administered group.
Sample, BCCA operated and SRe containing 100㎎/㎏/day administered group.
* significantly different when compared($p<0.05$).

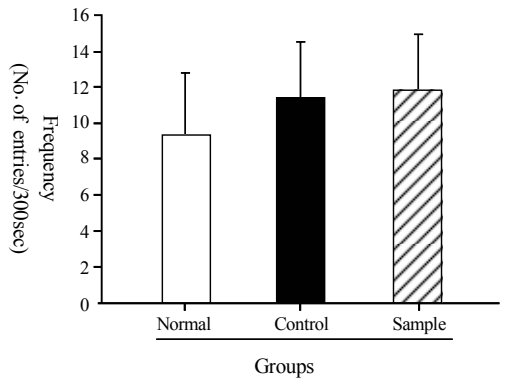

Fig. 4. Effects of SRe on the BCCA surgery on the number of arm entries into the closed arms of the elevated plus-maze test in rats. Values represent mean±S.E. of six rats per group.

3. Level of GSH and lipid peroxidation in brain homogenate

To gauge glutathione levels in the brain, GSH was determined in the striatum of BCCA operation applied rats. BCCA operation caused GSH reduction in brain regions examined. As shown in Fig. 5, there was a 50% decrease in GSH level when compared with the normal rats. But when SRe were administered for five days with the concentration of 100 ㎎/㎏, GSH activity significantly increased(Fig. 5).

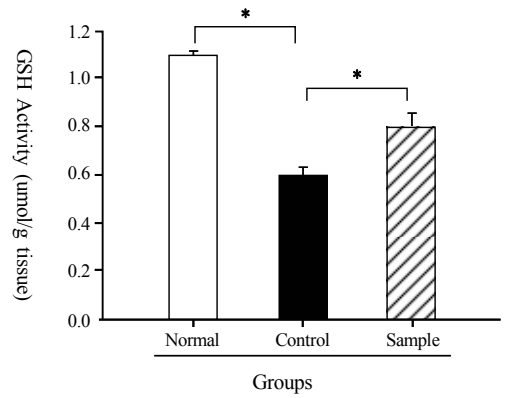

Fig. 5. The levels of reduced glutathione(GSH) in rat regional brain tissues. GSH was measured in striatum of rats.

* significantly different when compared($p<0.05$).

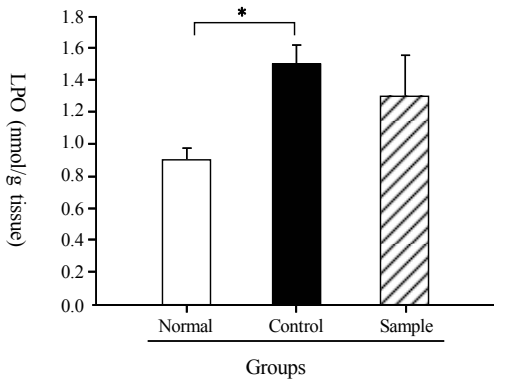

Fig. 6. The levels of lipid peroxide(LPO) in rat brain tissues. LPO was measured in striatum of rats.

* significantly different when compared($p<0.05$).

To evaluate the possible consequences of oxidative stress in the brain, lipid peroxide was measured in brain. There were significant increases in LPO production in striatum compared with the respective regions in normal rats. But SRe administeration showed no significant change(Fig. 6).

4. Effect on plasma catalase(CAT) activity

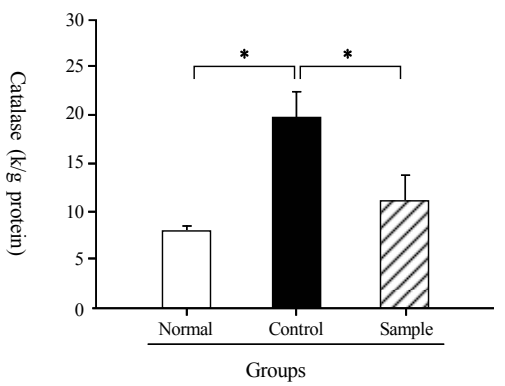

Fig. 7. CAT was measured in plasma of rats.

* significantly different when compared($p<0.05$).

The activities of CAT in control group were higher than in normal group, but when SRe were administered for five days with the concentration of 100 ㎎/㎏, CAT in plasma activated. The numerical values of normal, control and sample were 7.2±0.4, 18.6±2.4 and 11.3±3.4 k/g protein(Fig. 7).

5. Measurement of plasma Cu, Zn-SOD activity

The activity in all the groups were not statistically different. But control group showed increasing tendency compared with normal group. SRe administered sample group showed no difference. The numerical values of normal, control and sample were 12047± 638, 14302±2373 and 15295±1503 U/g protein(Fig. 8).

6. Measurement of plasma GSH

GSH level in normal group was higher than in control group. When SRe were administered for five days with the concentration of 100 ㎎/㎏, GSH in plasma activated. The numerical values of normal, control and sample were 1.7±0.4, 0.6±0.2 and 1.2± 0.2㎎/g protein(Fig. 9).

7. Measurement of plasma lipid peroxidation

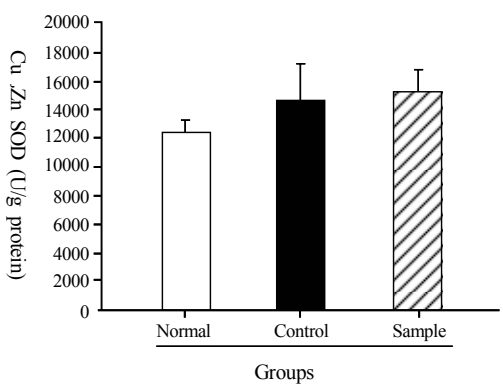

Fig. 8. Cu, Zn SOD was measured in plasma of rats.
* significantly different when compared.

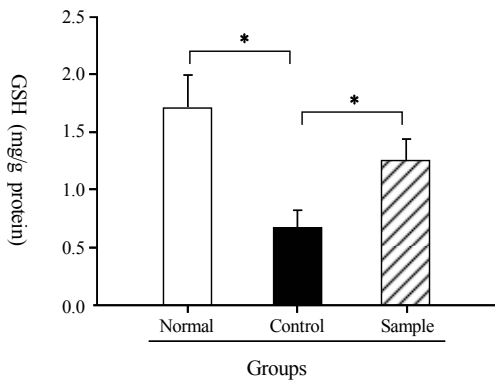

Fig. 9. GSH was measured in plasma of rats.
* significantly different when compared($p<0.05$).

SRe administration showed no significant changes on brain homogenate lipid peroxidation as appearing in Fig. 6, and the same with serum level. The numerical values of normal, control and sample were 3.7±0.8, 4.1±0.6 and 3.9±0.6 pmole/㎎ protein(Fig. 10).

8. Effect of SRe against NMDA induced neurotoxicity

The effect of SRe against NMDA induced neurotoxicity was evaluated by measuring the LDH activity in neuronal cells which were treated with 30 μM

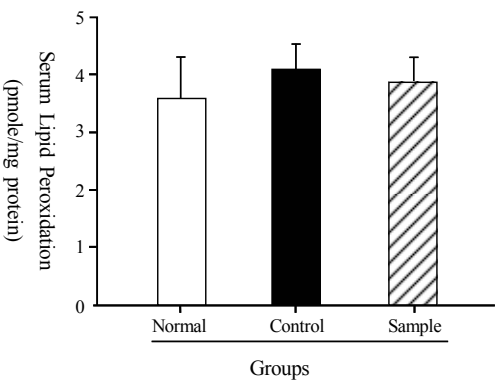

Fig. 10. Lipid peroxidation was measured in plasma of rats.
* significantly different when compared.

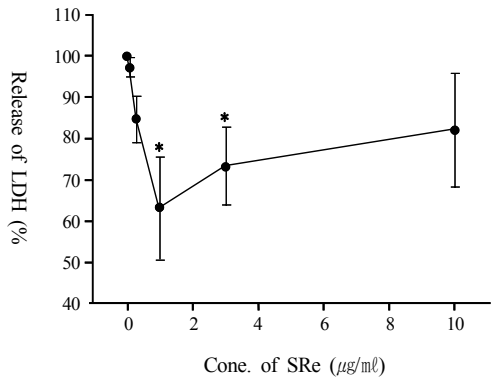

Fig. 11. Effect of SRe on the LDH activity in neuronal cells. All the groups were treated with 30 μM NMDA. The present data were expressed as mean±SE which were the % of control.

* statistically significant compared with control group($p<0.5$).

NMDA. When 1 and 3 ㎍/㎖ SRe was applied to the cultures, the quantities of LDH was significantly reduced when compared with cultures treated only with NMDA(Fig. 10). These results show that SRe has neuroprotective effect against NMDA neurotoxicity.

Discussion

Stroke has been ranked third most common cause of death worldwide and cerebrovascular diseases are considered second most frequent causes of projected deaths in the year 2020[15]. At the present state of knowledge treatment of ischemic brain injury is far from adequate[16]. And natural products, especially the Oriental herbal medicine, probably represent an ideal source to develop safe and effective agents for management of stroke.

Oxidative stress, which results from an imbalance between the generation and removal of reactive oxygen species, probably plays an important role in the development of tissue damage induced by arterial occlusion with subsequent reperfusion[17].

The radix of *Scutellaria baicalensis Georgi* is a traditional herb medicine, which is used as anti-inflammatory agent and smooth muscle relaxer, and it is also used as a constitute of some hepato-protective herb mixture such as Soshiho-tang(小柴胡湯)[18]. It was confirmed that the active components of which are flavonoids, baicalin, baicalein and wogonin are three major flavonoids of the root of *Scutellaria baicalensis Georgi*.

In the present study, the effects of *Scutellaria Radix* extract(SRe) were tested for the values of rCBF and PAD with Laser-Doppler Flowmetry(LDF) on ischemia/reperfusion induced brain hemodynamic change. LDF allows for real time, noninvasive, continuous recordings of local CBF. The LDF method has been widely used to trace hemodynamic changes in the superficial or the deep brain structures in experimental stroke research[19]. It has also been employed as a useful tool for imaging the instantaneous changes of cortical CBF related to cortical spreading depression or tracing stimulation elicited by a local vascular response[20]. The present study thus was carried out to determine the mechanism of action of SRe on rCBF by using LDF method.

For the ischemia/reperfusion induction, middle cerebral artery occlusion(MCAO) was carried out according to the method of Zea-Longa et al.. SRe treatment showed no significant alteration on cerebral hemodynamics of cerebral ischemia/reperfusion(Fig. 1), and the same on the cerebral blood vessel diameter by increasing PAD(Fig. 2).

When BCCA operation SRe five days administration of 100 ㎎/㎏ concentration applied to rats, SRe treatment showed an anxiolytic-like effect by extending latency time(Fig. 3), but on the moving frequency, SRe treated rats showed no significant change(Fig. 4).

The production of reactive oxygen species and reactive nitrogen species in excess of cellular antioxidant capabilities has been implicated in ischemic/reperfusion pathology[21], a variety of neurological disorders[22] and cardiovascular diseases[23]. The experimental induction of oxidative and nitrosative stress

by ischemia/reperfusion or neurotoxins offers paradigms suited to establishing the function of endogenous non-enzymatic antioxidants(such as ascorbate (AA), glutathione (GSH) and urate) and endogenous indicators of oxidative stress, such as urate, during pathological metabolic insult[24].

Manoli et al. and Baek et al. concluded in their study that the vulnerability to oxidative stress in the brain is region specific. Besides these, there are several studies, which investigate the stress-induced oxidative modifications in whole brain[25]. And in order to neutralize ROS, the body uses enzymatic (Cu,Zn-SOD, CAT) and non-enzymatic(GSH) antioxidants.

On the basis of above studies, oxidative stress concerned parametersl in brain and plasma were measured in the present study. That is to say, free radical scavengers like glutathione, superoxide dismutase and catalase. Lipid peroxides also measures. Quantification of lipid peroxidation is widely used to indicate oxidative injury in diseases. Lipid peroxidation is a complex process, and polyunsaturated fatty acids are readily susceptible to autoxidation.

GSH was determined in the striatum of BCCA operation applied rats. When SRe were administered for five days with the concentration of 100 ㎎/㎏, GSH activity significantly increased(Fig. 5). But SRe administeration showed no significant change in lipid peroxidation(Fig. 6).

When the activities of CAT, Cu, Zn-SOD and GSH were measured, CAT and GSH were activated by SRe administration(Fig. 7, 8, 9). And SRe administration showed no significant changes on brain homogenate lipid peroxidation as appearing in Fig. 6, and the same with serum level(Fig. 10).

Glutamate is the major excitatory neurotransmitter in the vertebrate brain and retina, but overactivation of glutamate receptors under pathophysiological conditions leads to neuronal death through a process termed "excitotoxicity"[26]. Pathological activation of glutamate receptors is thought to be a final common pathway leading to neuronal damage in a wide variety of neurological diseases[27]. Excessive influx of extracellular Ca^{2+} into neuronal cytoplasm via NMDA subtype of glutamate receptors leads to a series of potentially neurotoxic events.

The effect of SRe against NMDA induced neurotoxicity was evaluated by measuring the LDH activity in neuronal cells which were treated with 30 μM NMDA. When 1 and 3 ㎍/㎖ SRe was applied to the cultures, the quantities of LDH was significantly reduced when compared with cultures treated only with NMDA(Fig. 11).

Natural products(i.e. Oriental medicines) with such properties constitute an ideal choice for maximum therapeutic effect with minimal risk of iatrogenic adverse effects. Through this study, it can be concluded that the ischemia/reperfusion induced brain stress may have contributed to cerebral damage in rats, and the present study provides clear evidence for the beneficial effect of SRe on ischemia induced brain injury.

Conclusion

This study was carried out to determine whether SRe extract exerts beneficial effect against ischemia induced brain injury.

The results are as follows;

1. When SRe were administered for five days with the concentration of 100 ㎎/㎏, GSH activity significantly increased. But SRe administeration showed no significant change in lipid peroxidation.
2. When the activities of CAT, Cu, Zn-SOD and GSH were measured, CAT and GSH were activated by SRe administration.
3. When 1 and 3 ㎍/㎖ SRe was applied to the neuronal cell cultures, the quantities of LDH was significantly reduced when compared with cultures treated only with NMDA.

It can be concluded that the ischemia/reperfusion induced brain stress may have contributed to cerebral

damage in rats, and the present study provides clear evidence for the beneficial effect of SRe on ischemia induced brain injury.

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