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Original Article

Neurological Effects of *Bojungikki-tang and Bojungikki-tang-gamibang* on Focal Cerebral Ischemia of the MCAO Rats

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Objectives: This study demonstrates the neurological effects of *Bojungikki-tang and Bojungikki-tang-gamibang* on the focal cerebral ischemia of rats with ischemic damage caused by middle cerebral artery occlusion (MCAO).
Methods: Rats were treated with *Bojungikki-tang and Bojungikki-tang-gamibang* extracts for about five days after MCAO, and the size and volume of cerebral infarction and the ratio of cerebral edema were observed. From the immunohistochemical view, significant changes of outbreak of Bax, Bcl-2, c-Fos, HSP72, and iNOS were observed in the brain tissues.
Results: *Bojungikki-tang* repressed only brain edema and iNOS revelation led by focal cerebral ischemia, when considering significance. In contrast, *Bojungikki-tang-gamibang* demonstrated significant suppression of cerebral infarction, brain edema, Bax, c-Fos, HSP72, and iNOS induced by focal cerebral ischemia.
Conclusions: *Bojungikki-tang* is considered functional treatment for cerebral ischemic damage; it can be effective to relieve secondary brain edema and immune response. *Bojungikki-tang-gamibang* can have a direct function to alleviate brain infarct and to control the natural death of nerve cells which cerebral ischemic damage brings about.

Key Words : Neurological effects, *Bojungikki-tang (Bu xhong yi qi tang)*, *Bojungikki-tang-gamibang (Jia wei Bu xhong yi qi tang)*, focal cerebral ischemia, MCAO rats

Introduction

Stroke is a cerebrovascular disease, indicating a local neurological loss caused by an obstruction of blood circulation in the brain. It is defined by symptoms such as neurological deficits and dysarthria[1]. In the western medical science, they are known as cerebral infarction, cerebral hemorrhage, T.I.A, and hypertensive encephalopathy[2].

The main causes of the disease in oriental medicine are internal wind, fire heat, dampness phlegm, blood stasis, and deficiency syndrome[2]. Li Gao[3] from the Jin Dynasty in ancient China first compounded *Bojungikki-tang* (BJT). He stated that factors such as aging, mental problems or physical constitution led to *qi* deficiency, which brought an attack of the disease. BJT is a common prescription for irregular eating pattern, exhaustion of primary *qi*, the spleen and stomach *qi* decline and yang subsidence[3]. It also has been prescribed to treat other various diseases linked to *qi* decline[4], and studied widely in many cases as well[5-8].

In addition, *Bojungikki-tang-gamibang* (BJG) was made by adding more ingredients to BJT to strengthen

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its special effect that simulates the circulation of blood.

Some prescriptions for cerebral ischemia such as Hyulbuchuko-tang and Chungsimyuonja-eum have been studied widely[9-13]. However, there were few studies dealing with BJG[14]'s effect on stroke led by *qi* deficiency, nor the neurological differences between BJT and BJG.

This study was done with rats which were operated on by middle cerebral artery occlusion (MCAO) to cause ischemic damage. They were treated with BJT or BJG extracts, and the size and volume of cerebral infarction and the ratio of cerebral edema were observed. Throughout the observation from the immunohistochemical view, significant effects on the outbreak of Bax, Bcl-2, c-Fos, HSP72, and iNOS were carried out in the brain tissue. The results and the details supporting them are presented here.

Materials and Methods

1. Subjects

The subjects were Sprague-Dawley male rats of about 10 weeks age, purchased from Biogeomics Co. They weighed about 280 g. Their cage was maintained at temperature between 21~23 ℃, humidity between 40~60% and controlled lights switching on and off every 12 hours automatically. Aseptic food and water were provided freely. The objects were used after having settled down for a week in the laboratory.

Table 1. Contents of BJT and BJG

Name of formula	Scientific name	Dosage
BJT	*Astragalus membranaceus Bunge*	6.0 g
	Panax ginseng Ness.	4.0 g
	Atractylodes macrocephala Koidz.	4.0 g
	Glycyrrhiza uralensis Fisch.	4.0 g
	Angelica gigas Nakai	2.0 g
	Citrus unshiu Marcor.	2.0 g
	Cimicifuga heracleifolia Kom.	1.2 g
	Bupleurum chinese DC.	1.2 g
	Total	24.4 g
BJG	*Astragalus membranaceus Bunge*	6.0 g
	Panax ginseng Ness.	4.0 g
	Atractylodes macrocephala Koidz.	4.0 g
	Glycyrrhiza uralensis Fisch.	4.0 g
	Saposhnikovia divaricata Schiskin	4.0 g
	Ostericum koreanum Kitagawa.	4.0 g
	Gastrodia elata Bl.	4.0 g
	Pinellia ternata Breit.	4.0 g
	Arisaema japonicum Bl.	4.0 g
	Saussurea lappa Clarke	4.0 g
	Angelica gigas Nakai	2.0 g
	Citrus unshiu Marcor.	2.0 g
	Cimicifuga heracleifolia Kom.	1.2 g
	Bupleurum chinese DC.	1.2 g
	Total	48.4 g

2. Preparation and medication of the formula

BJT[21] and BJG[14] were used as prescription, and the ingredients are stated in Table 1. 244 g (10 packages) of BJT and 484 g (10 packages) of BJG were boiled in distilled water. After filtering them, vacuum evaporation and freeze drying were carried out obtaining powdered extracts of 82.2 g and 138.4 g from them, respectively. The dosage each time was 100 mg per 100 g of each rat's body weigh in order to compare the results of the same dosage of each formula. The first dose was given three hours after they awoke from anesthesia. Afterwards, each dose was given daily.

3. Separation of experimental groups

The rats were divided into four groups: Sham, Control, Sample-I and Sample-II groups; 12 rats were designated to each group. Except for the Sham group, they all received middle cerebral artery occlusion (MCAO). Among them, Sample-I was treated with BJT exact orally, as was Sample-II by BJG extract. Five days after the operation and treatment, the brain tissues were removed for observation.

4. Induction of focal cerebral ischemia

This experiment used the MCAO method[15]. It was done by inserting a nylon suture into the common carotid artery. The origins of the middle cerebral artery of the rats were locked out causing focal cerebral ischemia. To anesthetize them, a mixture of 70% N_2O, 30% O_2 and 5% isoflurane was used to start the operation, and the density was maintained at 1.0~1.5% during the operation. The temperature was maintained at 37.0±0.5 ℃ by a feedback-regulated heating pad throughout the operation. After opening the skin of the anterior neck, the common carotid artery and the branch of the internal carotid artery and external carotid artery were revealed. The next step was making a hole in the external carotid artery to allow the nylon suture in, and then the common carotid artery was cut to block the bloodstream temporarily. At this state, a nylon suture (4-0 monofilament, Ethicon, Edinburgh, Scotland) was inserted slowly into the internal carotid artery up to about 20 mm, so that it could close the origin of the middle cerebral artery. The suture had been rounded out at the end to 0.3 mm diameter and coated with 0.1% poly-L-lysine on the day of operation, for better performance. With the suture left in the vein and the other side shown outside the seam on the skin, the anterior neck was closed and the rats came out of the anesthesia. Two hours after the occlusion, they were anesthetized again to restore the bloodstream. The suture outside the seam was pulled out, then the bloodstream was released. They came out of the anesthesia right away.

5. Measuring the size and volume of cerebral infarction and the ratio of cerebral edema

Five days after MCAO, pentobarbital sodium was injected deeply into their abdominal cavity. After cutting their necks, the brains were taken out to make the tissue in slides of 2 mm thick, by using brain matrix (ASI, USA) for the white rats. The slides were stained using 2% 2,3,5-triphenyltetrazolium chloride (TTC)[16] and photos were taken by digital camera. The software “Image J” from NIH helped measure the size of the cerebral infarction in each slide and calculated the total volume of the damaged parts. Alongside that, cerebral edema ratio was measured by comparing the sizes of two hemispheres, one of which was normal, and the other in which focal cerebral ischemia had been induced.

6. Process on the brain tissues

The rats were anesthetized by pentobarbital sodium injection in the abdominal cavity. With the chest opened, 0.05M phosphate buffered saline (PBS) and 4% paraformaldehyde entered the bloodstream through the heart. The brain was extracted and treated by a

24-hour post-fixation and sediment in sucrose solvent. The next process was to freeze the brain in dry ice-isopentane solvent at -40℃, then keep it in one at -80℃ before making the tissue slides. The thickness of the tissues slides for staining was 50 ㎛ in coronal section format, by cryocut.

7. Immunohistochemical staining

The brain tissues were washed in 0.05M PBS for five minutes, a total of three times and put in 1% H_2O_2 for about 10-15 minutes to let it respond. After another three times washing, they were put into a blocking solution made of PBS to which was added 10% normal horse serum (Vectastain, USA) and bovine serum albumin (Sigma, USA) for about an hour to have it respond. Followed by three more times of washing again, they were treated by primary antibody. Bax (AB7977, 1:500, rabbit polyclonal; ABCAM, USA), Bcl-2 (SC7382, 1:500, mouse monoclonal; Santa Cruz, USA), c-fos (AB7963, 1:500, rabbit polyclonal; ABCAM, USA), HSP72 (HSP01, 1:500, mouse monoclonal; Oncogene, USA), and iNOS (610329, 1:250, mouse monoclonal; BD, USA) were the primary antibodies used for each, which had been thinned by PBS and Triton X-100 and left overnight at 4 ℃ for reaction[17]. After that, they were washed by PBS and left to respond for an hour, according to avidin-biotin immunoperoxidase method (ABC Vectastain Kit). Then diaminobenzidine tetrachloride (Sigma, USA) was used to form a color response, and the tissues were attached to the poly-L-lysine coated slides. After drying them for about two or three hours, dehydrating and suturing them were the last steps to making the tissue samples.

8. The observation on the result of the immunohistochemical staining

In order to quantitatively observe changes of Bax, Bcl-2, c-Fos, HSP72, and iNos, the images of the stained brain tissues were taken by CCD camera and stored in the image analyzing system. NIH "Image J" software measured the number of the positive cells in the MCAO operated cortex penumbra in the cerebral hemisphere. Each measurement supposed the same size for each tissue. Each section was 100,000 μm^2 for counting the cells.

9. Analysis of the statistics

Student's t-test method was used for inspection under conditions of $p<0.05$ on Sample-I and Sample-II against the Control group in analyzing the data.

Results

1. Changes of size of cerebral infarction area

An observation on the TTC-stained brain tissues showed that the Sham group did not have an area with brain infarction. Six brain tissue slides from the Control group indicated areas of brain infarction: 30.2±1.5 mm^2, 38.4±2.1 mm^2, 42.3±1.8 mm^2, 41.8±1.9 mm^2, 37.4±1.9 mm^2 and 25.5±1.4 mm^2.

It was 31.3±1.7 mm^2, 37.6±1.8 mm^2, 39.7±1.9 mm^2, 37.7±1.8 mm^2, 32.8±1.8 mm^2 and 23.1±1.2 mm^2 in the Sample-I group which had been treated with BJT. Though these data show moderate decreases, statistically, it was not a significant change.

On the other hand, the fourth and fifth slides from Sample-II group which had been treated with BJG showed significant declines in the condition of $p<0.05$; they were 27.5±1.6 mm^2, 36.2±2.0 mm^2, 38.6±1.7 mm^2, 36.0±1.7 mm^2, 30.1±1.8 mm^2 and 22.5±1.3 mm^2 (Fig. 1, 2).

2. Changes of volume of brain infarction

The Sham group did not show any area of brain infarction, while the Control group gave 431.2±11.3 mm^3 for brain infarct volume. The decrease of brain infarction volume in the Sample-I group was 404.4±10.7 mm^3, which was 6.2% compared with the Control group. However, this number was not

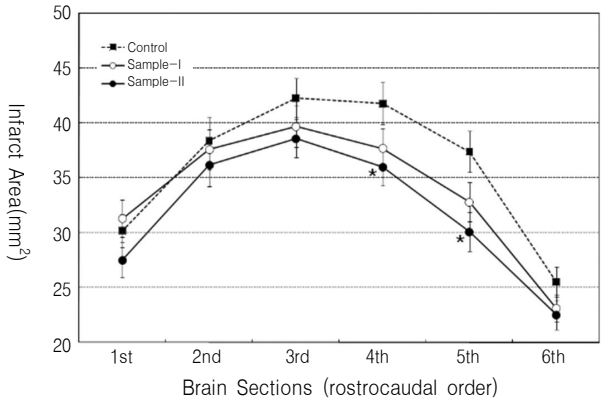

Fig. 1. The effect of BJT and BJG on TTC-stained infarct area in brain sections of the MCAO rats.
Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated significant (*; $p<0.05$) reduction of the infarct area in 4th and 5th brain sections as compared with Control.
Data presented as mean ± standard error.

included due to not falling into the proper standard range.

A significant result appeared in Sample-II group. The data indicated a great decline of brain infarct volume. As seen in Fig. 3, the drop was 381.8±10.2 mm^3, a decrease of 11.5%, gaining validity in the condition of $p<0.01$(Fig. 3).

3. Ratio changes of brain edema

An observation on the brain tissue slides showed that the Sham group did not show brain edema, while the Control group did at 132.8±3.4%. The Sample-I group did at 121.6±3.3%, which was a decline of 8.4% in relation to the Control group, attaining significance regarding $p<0.05$. As well as

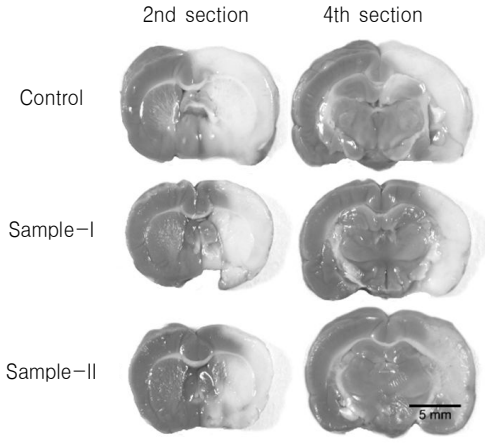

Fig. 2. TTC-stained 2nd and 4th brain sections of the MCAO rats.
Red colored regions are intact and white colored regions indicate infarct area.
Sample-I (BJT) group was not different from Control in 2nd and 4th brain sections. Sample-II (BJG) group demonstrated significant decrease of the infarct area in 4th brain sections as compared with Control, but not in 2nd brain section.

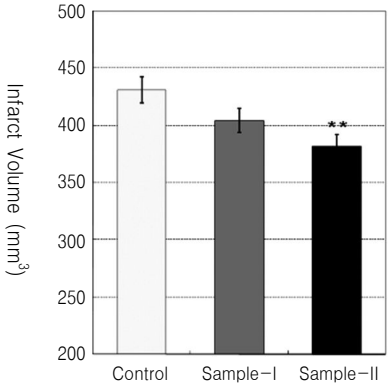

Fig. 3. The effect of BJT and BJG on TTC-stained total infarct volume of the MCAO rats.
Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated significant (**; p<0.01) reduction of total infarct volume as compared with Control.
Data presented as mean ± standard error.

Sample-I, the Sample-II group did at 118.7±2.6%, with a drop of 10.6%, under the same condition of p<0.01(Fig 4).

4. Changes of Bax revelation

In the penumbra of brain infarction, the nerve cells responding positive to Bax were counted. As seen in Fig. 5, the Sham group indicated 3.5±0.3 cells positive to Bax. It was 34.8±2.3 in the Control group, which implied focal cerebral ischemia had resulted in the great increase of Bax positive nerve cells.

In the Sample-I group, 27.9±2.5 cells responded positive to Bax. This was a decrease of 19.4% of positive cells; however, it was discarded because it did not meet the proper standard range. Meanwhile, the Sample-II group showed a decrease of 19.8±2.2 cells, which meant 43.1% cells had reduced. This is

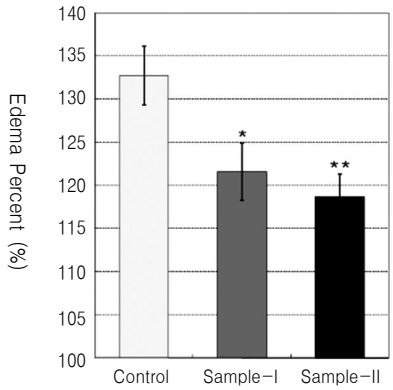

Fig. 4. The effect of BJT and BJG on edema percent of cerebral hemisphere of the MCAO rats.
Sample-I (BJT) and Sample-II (BJG) groups demonstrated significant (*; p<0.05, **; p<0.01) reduction of edema percent of cerebral hemisphere as compared with Control.
Data presented as mean ± standard error.

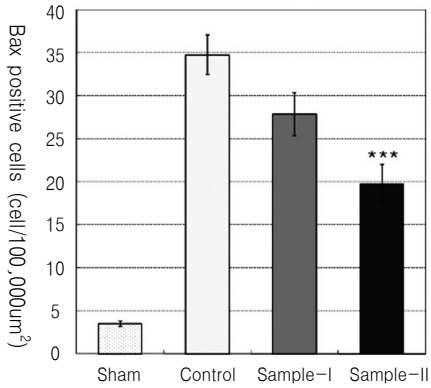

Fig. 5. The effect of BJT and BJG on Bax positive cells in cerebral penumbra of the MCAO rats.
Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated significant (***; p<0.001) decrease of Bax positive cells as compared with Control.
Data presented as mean ± standard error.

significant when considering the condition of p< 0.001(Fig. 5, 6).

5. Changes of Bcl-2 revelation

According to the result taken from the penumbra of cerebral infarction, the Sham group presented 5.2±0.8 Bcl-2 positive nerve cells; the Control group 18.6±1.8, which meant focal cerebral infarction brought about an increase of Bcl-2 positive nerve cells.

The Sample-I group also showed 19.1±1.7 positive cells, however it was statistically irrelevant. For the same reason, the number that Sample-II group was statistically not significant, even though 23.3±2.1 cells were counted positive which was 25.3% greater than the Control group (Fig. 7, 8).

6. Changes of c-Fos

In the penumbra of brain infarction, 17.5±2.3 nerve cells from the Sham group were counted positive

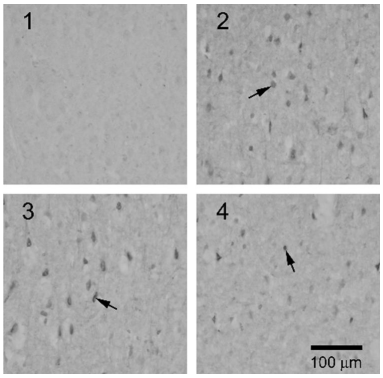

Fig. 6. Representative sections of Bax immuno-labeling in cerebral penumbra of the MCAO rats (1, Sham; 2, Control; 3, Sample-I; 4, Sample-II). Arrows indicate Bax positive cells.
Sample-I (BJT) group was not different from Control. Sample-II (BJG) group demonstrated significant decrease of Bax positive cells as compared with Control.

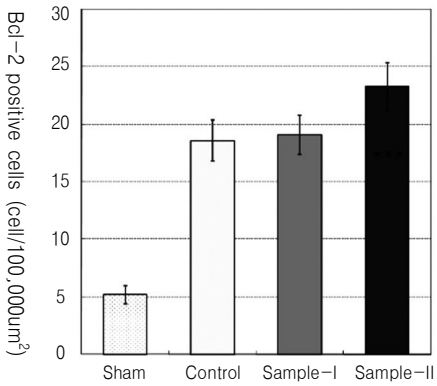

Fig. 7. The effect of BJT and BJG on Bcl-2 positive cells in cerebral penumbra of the MCAO rats.

Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated increase of Bcl-2 positive cells, but it was not significant statistically as compared with Control.
Data presented as mean ± standard error.

to c-Fos. It was 63.6±7.1 in the Control group, which meant focal cerebral infarction caused a great increase of c-Fos positive cells.

The Sample-I group displayed a decline of 23.7%, which implied c-Fos positive cells had been reduced by 48.5±5.3. However, this number was statistically insignificant. The Sample-II group showed a decrease of 31.9%. In other words, only 43.3±4.8 cells were left positive, which was included under the condition of $p<0.05$(Fig. 9, 10).

7. Changes of HSP72 revelation

In the same way, from the penumbra of brain infarct, certain changes were observed. The Sham group displayed 9.3±1.7 HSP72 positive nerve cells, and 71.5±8.3 nerve cells were counted in the Control group. This also showed that focal cerebral infarction resulted in a huge increase of HSP72 positive cells.

The Sample-I group presented 50.8±6.2, which showed 29.0% of positive cells were reduced in relation to the Control group; however, it was not a

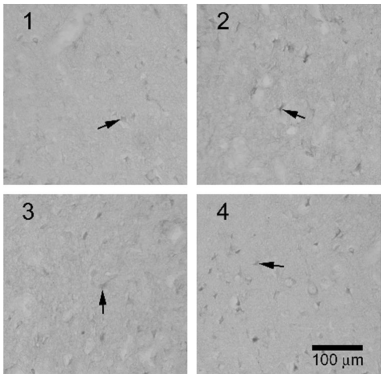

Fig. 8. Representative sections of Bcl-2 immuno-labeling in cerebral penumbra of the MCAO rats (1, Sham; 2, Control; 3, Sample-I; 4, Sample-II). Arrows indicate Bcl-2 positive cells.

Sample-I (BJT) group was not different from Control. Sample-II (BJG) group demonstrated increase of Bcl-2 positive cells, but it was not significant statistically as compared with Control.

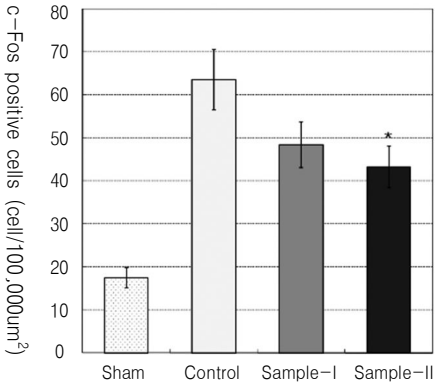

Fig. 9. The effect of BJT and BJG on c-Fos positive cells in cerebral penumbra of the MCAO rats.
Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated significant (*; $p<0.05$) decrease of c-Fos positive cells as compared with Control.
Data presented as mean ± standard error.

significant change statistically. The Sample-II group indicated a significant change; the number of HSP72 positive cells was 42.5±4.4. This was 40.4% decrease compared to the Control group, which was certainly significant in the condition of $p<0.05$(Fig. 11, 12).

8. Changes of iNOS revelation

Measuring the numbers of iNOS positive nerve cells in the penumbra of brain infarct area, the Sham group showed 1.2±0.2 iNOS positive cells. The Control group did 34.6±2.4, which meant focal cerebral infarction caused an increase of iNOS positive cells.

In terms of iNOS positive nerve cells, both the Sample-I and Sample-II groups indicated significant results: 24.5±1.8 from the Sample-I group and 25.2±1.6 from the Sample-II group. The former meant 29.2% decline and the latter meant 27.2% decline compared to the Control group, both under the condition of $p<0.05$(Fig. 13, 14).

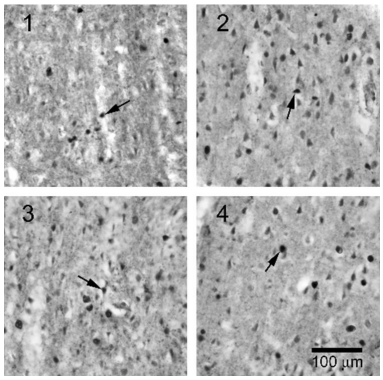

Fig. 10. Representative sections of c-Fos immuno-labeling in cerebral penumbra of the MCAO rats (1, Sham; 2, Control; 3, Sample-I; 4, Sample-II). Arrows indicate c-Fos positive cells.
Sample-I (BJT) group was not different from Control. Sample-II (BJG) group demonstrated significant decrease of c-Fos positive cells as compared with Control.

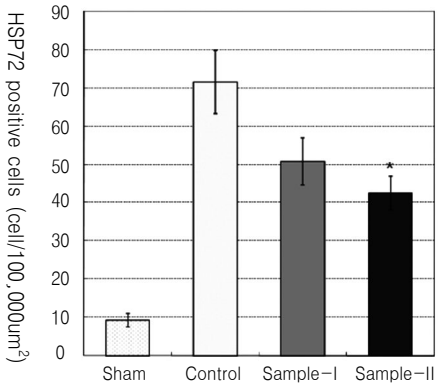

Fig. 11. The effect of BJT and BJG on HSP72 positive cells in cerebral penumbra of the MCAO rats.

Sample-I (BJT) group was statistically not different from Control. Sample-II (BJG) group demonstrated significant (*; $p<0.05$) decrease of HSP72 positive cells as compared with Control.
Data presented as mean ± standard error.

Discussion

According to 'the mortality statistics in 2007' released by the Korean National Statistical Office, cerebrovascular disease was the second most fatal disease following cancer. Today, it is dominant among people over 50[18] and even though it does not cause death right away, its serious sequelae cause both social and economic loss[19]. Hence, the major concern of the disease is how to minimize and recover the damaged nerve cells as well as to deal with sequelae in terms of recovery.

Cerebral ischemia is roughly categorized into global cerebral ischemia and focal cerebral ischemia. Clinically, most ischemic cerebrovascular diseases belong to the latter category. They are led by occluded internal carotid artery or middle cerebral artery. Middle cerebral artery originates from the branch of the internal carotid artery. Occluded middle cerebral artery induces hemiparesis, sensory disorder

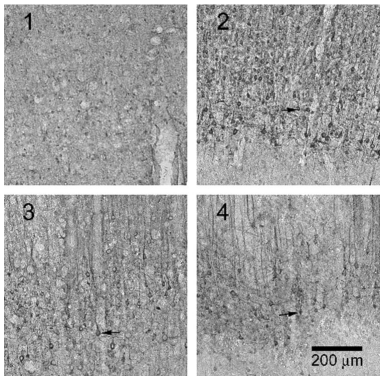

Fig. 12. Representative sections of HSP72 immuno-labeling in cerebral penumbra of the MCAO rats (1, Sham; 2, Control; 3, Sample-I; 4, Sample-II). Arrows indicate HSP72 positive cells.

Sample-I (BJT) group was not different from Control. Sample-II (BJG) group demonstrated significant decrease of HSP72 positive cells as compared with Control.

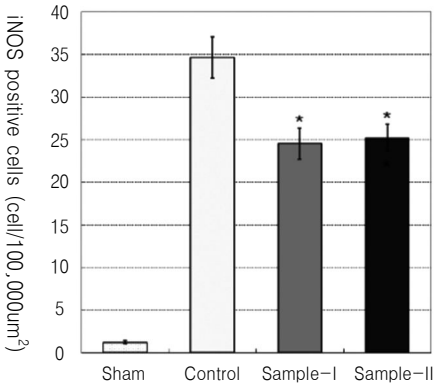

Fig. 13. The effect of BJT and BJG on iNOS positive cells in cerebral penumbra of the MCAO rats.
Sample-I (BJT) and Sample-II (BJG) groups demonstrated significant (*; $p<0.05$) decrease of iNOS positive cells as compared with Control.
Data presented as mean ± standard error.

in the opposite side of body and aphasia[1].

In Oriental medicine, it is said that internal wind, fire heat, phlegm, blood stasis and deficiency of *qi* and blood are the factors of stroke, so most of the treatments for stroke focus on removing those factors[20].

Especially, BJT may be prescribed for stroke caused by *qi* deficiency. It is one of the effective prescriptions historically. Historically, it first appeared in Biwiron written by Li Gao[3]. It is also a typical prescription for deficiency of middle *qi* and overall symptoms of *qi* deficiency[21]. This prescription may tonify the spleen and raise and circulate pure *qi*[21], which is effective against fever, heart discomfort, sweating, fatigue, dizziness, numbness, and weakness[21]. It has been prescribed for stroke or heart diseases, and even for gastritis, stomach ulcer, indigestion, womb disease, stress, and allergic rhinitis[22-25]. Thus far, many studies have investigated its effects on immunity and cancer[26,27].

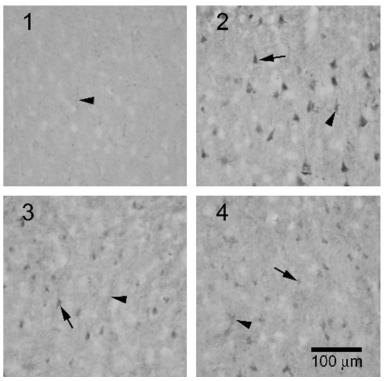

Fig. 14. Representative sections of iNOS immuno-labeling in cerebral penumbra of the MCAO rats (1, Sham; 2, Control; 3, Sample-I; 4, Sample-II). Arrows indicate iNOS positive neurons and arrow heads indicate glial cells.
Sample-I (BJT) and Sample-II groups demonstrated significant decrease of iNOS positive cells as compared with Control.

BJG in this research is found in *Kogeumshilhumbang*[14]. It is formulated by adding *Saposhnikovia divaricata* Schiskin, *Ostericum koreanum* Kitagawa., Gastrodia elata Bl., Pinellia ternata Breit., *Arisaema japonicum* Bl., and *Saussurea lappa* Clarke to BJT. It is clinically prescribed for stroke caused by *qi* deficiency.

The focal cerebral ischemia model from the rats was practically close enough to clinical stroke[17] to allow demonstrating the effects of BJT and BJG. Hence the sizes and volumes of cerebral infarction, the ratio of cerebral edema, changes of Bax, Bcl-2, c-Fos, HSP72, and iNOS revelation were observed from the immunohistochemical view.

According to TTC stained slides of the brain, the size of cerebral infarction of the Sample-I group declined compared to the Control. However, it did not do so in a statistically significant manner. The Sample-II group indicated significant decrease of the size of cerebral infarction in the fourth and fifth slides, in relation to the Control. In terms of volume of cerebral infarction, the Sample-I group displayed its decrease comparing to the Control, though it was not statistically significant. On the other hand, the result shown by the Sample-II group was a dramatic drop, which was statistically significant. Those results show the BJG may have a reliable effect on focal cerebral ischemia in protecting the nerve cells, in terms of histopathology.

Brain edema is generally caused by injuries in the brain, such as cerebral ischemia and brain trauma. It accelerates permeability of the capillaries and unsettles the blood-brain barriers (BBB). Then, water transfers from the vessel to the brain tissue, which finally worsens the troubles even up to death[28].

The ratio of the brain infarct area showed significant reduction of brain infarct size in the Sample-I and Sample-II groups. Especially, the Sample-II group displayed a greater decrease, in relation to both the Control and Sample-I groups. That means both BJT and BJG work well for reducing brain infarct while the latter is moderately more effective.

Bax, as well as Bcl-2, has control over the cell's natural death[29]. Their amino acid orders are very similar, however the functions differ. Bax is seen in the cells of the central nervous system. When its function exceeds, the cells face natural death. Bax tends to increase with aging. On the other hand, Bcl-2 prevents the cells from natural death and vitalizes the cells. It exists in the nerve cells during the growth period, and it decreases rapidly with aging. That positive function of Bcl-2 comes out by linking and blocking Bax's function. In other words, Bax and Bcl-2 are counterparts in terms of life and death of the cells[17]. Particularly, many reports show that Bax decides the possibility of damage in the nerve cells caused by many stimuli such as cerebral ischemia[30]. This means that Bax revelation may be used as an index to distinguish whether nerve cells would be sacrificed by cerebral ischemia[31].

In the penumbra of brain infarct, it drew a rapid growth of the number of Bax positive nerve cells which were triggered by focal cerebral ischemia. The number from the Sample-I group was discarded because of not falling into the proper standard range even though it was a certain decrease. In the Sample-II group, Bax positive nerve cells showed a significant reduction, compared to the Control. Therefore, BJG is effective in protecting the nerve cells from damage.

Also, the number of Bcl-2 positive nerve cells in penumbra of cerebral infarction showed an increase in accordance of focal cerebral ischemia. The Sample -I group indicated that Bcl-2 positive nerve cells increased in relation to the Control. However, it was statistically not significant. In the same way, the Sample-II group also displayed a statistically insignificant increase.

Growth of Bax revelation and decline of Bcl-2 revelation encourage the natural death of nerve cells and vice versa. This mechanism is important in terms of controlling natural death. However, the

main concern in dealing with the mechanism is the relative ratio of Bax and Bcl-2 revelation[32]. Therefore, BJG's effect on controlling Bax revelation may be significant regarding the ratio between them.

Cerebral ischemia causes an immediate change in the brain tissue in a biochemical way. Especially within several minutes after cerebral ischemia, revelation of the genes distinctively increase. Those kinds of genes are called immediate early genes (IEGs). c-Fos and c-Jun are the most common ones among them[33]. They stay calm and low at the normal state, and they appear in the corresponding brain sections when a biological stimulus is given[34]. On the other side, in some pathological conditions such as seizure or cerebral ischemia, IEG revelation tends to rise in broader areas of the central nervous system[35]. It manifests not only in the damaged part, but also the part surrounding the damage[36]. It is also reported to have a certain relationship with various mechanisms of cerebral ischemia, for instance, apoptotic cell death[37]. In general cases of light focal cerebral ischemia, its revelation is seen only inside the ischemic area. In more serious cases of focal cerebral ischemia, IEGs are found outside the ischemic area[37].

The increase of c-Fos positive nerve cells led by focal cerebral ischemia was sharp. The Sample-I group indicated a drop of c-Fos positive cells, which was statistically insignificant. In contrast, the Sample -II group drew a statistically significant decline of c-Fos positive nerve cells. This means that BJG may have certain control over cerebral ischemic damage.

HSP72 is one of the stress-induced proteins. Heat-shock gene revelation rises responding to all kinds of stress such as heat shock and ischemia, which generate metamorphic proteins[38]. It is found in all sorts of mammal cells, as well as plants, yeast and even bacteria[39]. Particularly, it is compounded by responding to various stresses like heat, heavy metals, toxins, and ischemia[40]. Later, it is combined with metamorphic proteins, and then it attempts to recover the enzyme function and the 3-dimensional structure of the metamorphic proteins. In other words, the metamorphic proteins stimulate HSP72 revelation to restore the structure and function of metamorphic proteins[41]. Hence HSP72 revelation outside the brain infarct area may be used to distinguish penumbra of cerebral ischemia or zones of protein denaturation in the brain infarct area[42].

The number of HSP72 positive nerve cells displayed a dramatic increase induced by focal cerebral ischemia. The Sample-I group again showed a statistically insignificant result compared to the Control, even though it declined. However, the result was statistically significant since in the Sample-II group, HSP72 positive nerve cells decreased in relation to the Control. This indicates BJG may shrink the brain infarct area.

The mechanism that cerebral ischemia causes brain edema is stated as follows: permeability of the brain blood vessels increases, and the vessel wall of the brain becomes slack. iNOS revelation responses to that process at this state[43]. iNOS is one of the enzymes which makes nitric oxide (NO). NO itself is inherently produced as a free radical. Its biological and pathological function varies. When its density rises, it takes part not only in cerebral infarction, but in cerebral ischemia, traumatic brain injury, and degenerative nerve disease as well[44]. Particularly, when NO is generated by iNOS, it is released for a long time, independently from the consistency of Ca^{2+} in the cell. It also grows the size of brain infarct caused by cerebral ischemia[44]. In addition, many studies indicate that NO is directly engaged in BBB damage which is caused by cerebral ischemia or inflammation[43]; BBB damage increases permeability of the brain vessel wall, which brings about brain tissue edema[45].

According to the observations on iNOS positive nerve cells, focal cerebral ischemia triggers an extreme growth of iNOS positive nerve cells. Both the Sample-I and Sample-II groups indicated significant declines of iNOS positive nerve cells, in

relation to the Control. Therefore, both BJT and BJG may prevent brain edema directly.

In this experiment, the sizes and volumes of brain infarct and the ratio of brain edema were observed on the fifth day after focal cerebral ischemia was induced. Observations on Bax, Bcl-2, c-Fos, HSP72, and iNOS revelation in the brain tissues were done by the immunohistochemical method. They revealed that BJT reduced brain edema ratio induced by focal cerebral ischemia and iNOS positive nerve cells with certain validity. Also, it tended to diminish the volume and size of cerebral infarction caused by focal cerebral ischemia, however it was not included regarding validity. On the other hand, it did not cut down the positive nerve cells such as Bax, Bcl-2, c-Fos, and HSP72, considering validity. Therefore, it is possible to say that BJT does not function directly against cerebral ischemic damage significantly; however, it may alleviate secondary brain edema and immune reaction.

BJG reduced the size of brain infarct in some of the brain tissue slides. It was also significantly effective to decrease brain infarct volume and brain edema ratio. Moreover, it was obvious that it prevented iNos positive nerve cells from growing and encouraged Bcl-2 revelation. Hence, BJG may have a direct function to suppress natural death of the nerve cells and relieving brain infarct, which are cause by cerebral ischemic damage.

More clinical studies about BJT and BJG's effects on stroke led by *qi* deficiency are expected.

Conclusion

BJT represses brain edema and iNOS revelation led by focal cerebral ischemia. This study implies that BJT can have a potentially weak function for cerebral ischemic damage; while also having a certain valid effect in relieving secondary brain edema and immune response.

In contrast, BJG demonstrates valid suppression on cerebral infarction, brain edema, Bax, c-Fos, HSP72, and iNOS induced by focal cerebral ischemia. Therefore, it may have a direct function to alleviate brain infarct and to control the natural death of the nerve cells caused by cerebral ischemia.

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