Protective Effect of Buckwheat Polyphenols Against Long-Lasting Impairment of Spatial Memory Associated With Hippocampal Neuronal Damage in Rats Subjected to Repeated Cerebral Ischemia

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Abstract. In the present experiment, we studied the action of buckwheat polyphenol (BWP, from Fagopyrum esculentum Moench) in a repeated cerebral ischemia model, which induced a strong and long-lasting impairment of spatial memory in 8-arm radial maze with hippocampal CA1 cell death in rats. BWP (600 mg/kg, continuous 21-day p.o.) significantly ameliorated not only the impairment of spatial memory in the 8-arm radial maze, but also necrosis and TUNEL-positive cells in the hippocampal CA1 area subjected to repeated cerebral ischemia (10 min × 2 times occlusion, 1-h interval) in rats. In order to investigate the mechanism of BWP protective action, we measured the release of glutamate and NOx (NO2⁻ + NO3⁻) production induced by repeated cerebral ischemia in the rat dorsal hippocampus using microdialysis. A 14-day BWP treatment significantly inhibited the excess release of glutamate after the second occlusion. In addition, the BWP remarkably suppressed a delayed increase in NOx induced by repeated cerebral ischemia in the dorsal hippocampus as determined in vivo by microdialysis. However, the 14-day treatment did not affect hippocampal blood flow in either intact rats or rats subjected to repeated ischemia measured by laser Doppler flowmeter. These results suggested that BWP might ameliorate spatial memory impairment by inhibiting glutamate release and the delayed generation of NOx in rats subjected to repeated cerebral ischemia.

Keywords: buckwheat polyphenol, repeated cerebral ischemia, spatial memory, glutamate, nitric oxide

Introduction

Cerebral ischemia causes irreversible neuronal degeneration in hippocampal CA1 neurons. Excess release of glutamate and subsequent Ca²⁺ entry during cerebral ischemia contribute to ischemic CA1 neuronal injury (1). Both N-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptor antagonists prevented the neuronal injury. Recently, it was shown that AMPA increased intracellular Ca²⁺, hydrogen peroxide and malondialdehyde levels through AMPA receptors in hippocampal neurons (2). We also found that post-treatment of an AMPA-receptor antagonist, YM-90K, but not a NMDA-receptor antagonist, MK-801, prevented the spatial memory impairment in the 8-arm radial maze and apoptosis in hippocampal CA1 in rats subjected to repeated ischemia (3). Nimodipine, a Ca²⁺ channel blocker, improved impairment of spatial memory induced by repeated cerebral ischemia in the 8-arm radial maze (4). Moreover, repeated ischemia resulted in a delayed increase of nitric oxide (NO) and post-ischemic administration of N⁴-nitro-L-arginine, a non-selective nitric oxide synthase (NOS) inhibitor, prevented both spatial memory impairments and hippocampal apoptosis induced by repeated ischemia (5). These results suggest that the
excess glutamate release/AMPA receptor/Ca$^{2+}$ influx /NO signaling pathway is important to the impairment of spatial memory and induction of hippocampal neuronal injury in rats subjected to repeated cerebral ischemia.

Studies have confirmed that polyphenolic compounds in tea and red wine can ameliorate the damage to neurons induced by cerebral ischemia by scavenging free radicals (6, 7). In this experiment, we focused on the action of buckwheat polyphenol (BWP) extracted from *Fagopyrum esculentum* Moench on neuronal injury induced by repeated cerebral ischemia. Buckwheat shows cholesterol-lowering effects (8), antihypertensive effects (9), antioxidant activities (10), and improves constipation and obesity. BWP contains many polyphenolic compounds such as catechin, epicatechin, rutin, hyperoside, quercetin, and oligomers of catechin and epicatechin. Catechin and epicatechin in tea exhibit multiple pharmacological actions such as, neuroprotective action (11), bactericidal action (12), hypcholesterolemic action (13), inhibition of angiotensin converting enzyme (14), antioxidation (15), and anti-HIV agent action (16). Rutin has been used in Chinese medicine as a remedy for hypertension and can inhibit oxidative damage induced by ultraviolet radiation (17). Quercetin in red wine is a versatile antioxidant and attenuates vacular formation in the optic tract in a rat chronic hypoperfusion model (18). Hyperoside has anti-inflammatory and spasmyotic activity (19) and enhances antioxidative capacity (20). It is reported that a 20-day p.o. treatment with BWP resulted in higher activity level of antioxidant enzymes such as superoxide dimustase, catalase, and glutathione peroxidase in a renal ischemia-reperfusion model (21). However, there is no report about the effects of BWP on cerebral ischemia. Therefore, we examined whether BWP could prevent the impairment of spatial memory and neuronal damage due to repeated cerebral ischemia. Furthermore, in order to determine the mechanism, we examined the action of BWP on glutamate release and the delayed increase of NO$\cdot$ production, which was regarded as the main cause of neuronal damage induced by repeated cerebral ischemia in rats.

**Materials and Methods**

**Drug and treatment**

Buckwheat polyphenol (polygonaecae, *Fagopyrum esculentum* Moench) was obtained from Amino Up Chemical Co., Ltd. (Sapporo). It contained about 25% polyphenol, of which oligomers of catechin and epicatechin accounted for about 80%, and other ingredients such as catechin (0.21%), epicatechin (0.04%), quercetin (0.01%), rutin (0.02%), and hyperoside (0.25%). It was dissolved in distilled water. The 21-day regimen with BWP (14-day pre-ischemic and 7-day post-ischemic administration) was given p.o. to rats in an 8-arm radial maze. In the experiment of NO$\cdot$, blood flow and glutamate release, 14-day pre-ischemic administration was given p.o. to rats.

**Animals**

Male Wistar rats weighing 250 – 300 g were obtained from the Kyu Do Co., Ltd. (Saga). They were housed in groups of 4 to 5 per cage in a room with controlled temperature (23 ± 2°C) and a relative humidity of 60 ± 10%, and maintained on a 12-h light-dark circle. All procedures regarding animal care and use were carried out based on the regulations dictated by the Experimental Animal Care and Use Committee of Fukuoka University.

**8-arm radial maze task**

Behavioral testing was conducted as previously reported (22) in an 8-arm radial maze (Neuroscience Co., Tokyo). The maze consisted of a central platform, 24 cm in diameter, with 8 arms extending radially. The rat was allowed to visit each arm to eat all 8 pellets in the food cups which were located near the end of each arm. Each test animal was trained once per day to memorize the apparatus. The performance of the test animals in each trial was assessed using three parameters: the number of correct choices in the initial 8 chosen arms, the number of errors which was defined as choosing arms that had already been visited, and the time that elapsed before the animal ate all 8 pellets. When the test animals made 7 or 8 correct choices and less than one error in three successive sessions, that is, the animals had memorized the maze, they could be used for the ischemic operation. The 21-day regimen with BWP (14-day pre-ischemic and 7-day post-ischemic administration) was given p.o. to the rats that acquired spatial memory, and vehicle (distilled water) was administered as the control group. The post-ischemic administration with BWP was given p.o. for 7 days after the occlusion.

**Cerebral ischemic treatment**

The test animals that had acquired spatial memory were tested according to the methods described by Chung et al. (23). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and were immobilized in a stereotaxic apparatus (Type SR-6; Narishige Scientific Instrument Lab., Tokyo). The bilateral arteries beneath the alar foramina of the first vertebra were electrocoagulated using a bipolar coagulator (MICRO-
3D; Mizuho Industrial Co., Tokyo), and both common carotid arteries were exposed bilaterally using a hydraulic pressure vascular occluder (OC: 1.5 mm diameter; Technical Supply Co., Osaka) applied to each exposed artery. On the following day, the common carotid arteries were compressed with the hydraulic pressure occluders operated by means of a water-filled syringe, and the cerebral circulation was interrupted for 10 min 2 times in 1 h. Any rat that failed to demonstrate a loss of righting reflex during arterial occlusion was excluded from the subsequent experiment. Rats that underwent only cauterization of the vertebral arteries and were then fitted with the occluders on the common carotid artery were used as sham-operated controls.

**Histologic examination**

The rats were sacrificed after the behavior test. They were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused transcardially with cold heparinized saline followed by 4% paraformaldehyde. The brains were removed from the skull and postfixed overnight in paraformaldehyde. The fixed brains were dehydrated and embedded in paraffin. Representative coronal sections (5-μm-thick) including the dorsal hippocampus were obtained with a rotary microtome. Tissue sections were stained with HE stain (hematoxylin and eosin) and TUNEL stain. In HE-staining, they were examined with a light microscope and the neuronal damage in the hippocampus was evaluated by determining the neuronal density of the CA1 neurons per 1 mm linear length of the hippocampus as described above in the measurement of glutamate and housed in a single cage (2 days following the surgery). The hippocampus of rats were perfused and sliced at a thickness of 40 μm. The injected sites were analyzed.

**Measurement of glutamate release**

Brain microdialysis was performed as described above. The guide cannula and microdialysis probes (Eicom) were just as the same as described previously. The rats were implanted with the guide cannula that was placed in the coordinates of the dorsal hippocampus as described above in the measurement of glutamate and housed in a single cage (2 days following the surgery). The hippocampus of rats were perfused with Ringer’s solution (as above) at a rate of 2 μL/min by means of a microinjector pump (ESP-64, Eicom). The syringe was connected to the probe inlet by polyethylene tubing; the probe outlet was connected to the sample loop of the analytical system by polyethylene tubing, and NO metabolites were separated on a reverse-phase separation column (E-ENZ, 3 x 4 mm; Eicom) with the reverse-phase (15 mM phosphate buffer, pH 7.4), 2-ketoglutaric acid and NH$_4^+$ in the enzyme column (E-ENZ, 3 x 4 mm) which was fitted with l-glutamate oxidase and the reaction column oven was set at 30°C (ATC-300, Eicom). When the electrode (ECD-Pt, pH 7.5) had a applied voltage of 500 mV vs Ag/AgCl, H$_2$O$_2$ was oxidized into oxygen (O$_2$), 2H$^+$, and 2e$^-; and the electrons were measured by an electrochemical detector (ECD-300, Eicom). The maximum of electric current was examined 7 min after measurement started. After completion of the experiment, the animals were injected with 2 μL cresyl violet dye to confirm the cannula placement. Ten minutes after this microinjection, the animals were anesthetized with ether and decapitated. After the brain was removed, it was frozen and sliced at a thickness of 40 μm. The injected sites were confirmed by macroscopic examination. Data only from animals in which the injections were made into the desired sites were analyzed.

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in a reduction column packed with copper-plated cadmium filings (NO-RED, Eicom). NO\textsuperscript{2−} was mixed with a Griess reagent to form a purple azo dye in a reaction coil. The separation and reaction columns and the reaction coil were placed in a column oven set at 35°C. The absorbance of the color of the product dye at 540 nm was measured with a flow-through spectrophotometer (NOD-10, Eicom). The mobile phase consisted of 10% methanol containing 0.15 M NaCl-NH\textsubscript{4}Cl and 0.5 g/L of 4Na-EDTA. The Griess reagent, which was 1.25% HCl containing 5 g of NO\textsubscript{3} plus NO\textsubscript{2} in Ringer’s solution and the reliability of the reduction column were examined in each experiment. The NO\textsubscript{3} concentrations (NO\textsubscript{2} plus NO\textsubscript{3}) were expressed as the percentage of the average pre-fraction. After the experiment finished, the confirmation of cannula and the data analysis were performed as described for the measurement of glutamate.

**Measurement of hippocampal blood flow**

Hippocampal blood flow was measured using laser Doppler flowmetry (TFB-LC1; Unique Medical, Tokyo). A laser Doppler flowmetry probe (Needle Type ON97-066, tip diameter 500 µm; Unique Medical) was inserted into the left dorsal hippocampus (A: −3.8, L: 2.0, V: 2.0). Hippocampal blood flow was expressed as a percentage taking the mean baseline blood flow as 100%.

**Measurement of blood pressure**

Systolic blood pressure was measured with the tail-cuff method at 8 to 10 weeks of age using a BP Monitor MK-1100 (Muromachi Kikai Co., Ltd., Tokyo). The rats were placed in a nontransparent plastic cage that allowed free but restricted movement. The rectal body temperature of the rats was measured and maintained at 37 – 38°C by placing the cage on a water-circulated heating pad. Experiments did not start until the resting heart rate of the rats was below 400 beats per minute and mean arterial blood pressure was <115 mmHg. Animals were awake and restrained by being lightly wrapped in a restraining device. At least 4 consecutive consistent readings were taken during 4 separate sessions, and the pressures were averaged to give a final blood pressure.

**Statistical analyses**

Values were expressed as means ± standard errors. The data on the behavioral test and CA1 pyramidal neurons were analyzed with the Mann-Whitney U test. The data on glutamate release, and blood flow were analyzed using Student’s t-test. NO metabolites were analyzed by repeated measures of two-way ANOVA followed by Tukey’s test for post-hoc analysis. Significance was considered at \( P<0.05 \).

**Results**

**Effect of the 21-day p.o. regimen with BWP on repeated ischemia-induced impairments of spatial memory in the 8-arm radial maze test**

In the 21-day pre-ischemic and post-ischemic administration, BWP (100 – 600 mg/kg) was administered p.o. for 14 continuous days and 10-min ischemia was carried out 2 times with a 1-h interval (repeated-ischemia); and then it was administered for a further 7 consecutive days before the retention test of the 8-arm radial maze was carried out. In the post-ischemic administration, BWP (600 mg/kg) was administered p.o. for 7 consecutive days after repeated ischemia. Repeated ischemia (vehicle-treated group) significantly decreased correct choices and increased errors (correct choices and errors, \( P<0.001 \), Fig. 1). The 21-day p.o. regimen with BWP (600 mg/kg) significantly improved spatial memory impairments induced by repeated ischemia in the 8-arm radial maze test (correct choices and errors, \( P<0.05 \), Fig. 1). However, the post-ischemic administration with BWP failed to improve them (data not shown).

**Effect of the 21-day p.o. regimen with BWP on the repeated ischemia-induced neuronal injuries in the hippocampal CA1 region**

In the vehicle-treated group of the repeated ischemia groups, the CA1 pyramidal neurons showed pyknosis, enosinophilia, karyorrhexia, and chromosome condensation in comparison with the sham group by HE-staining. Repeated ischemia induced cell damage in the hippocampal CA1 pyramidal neurons (\( P<0.001 \), Fig. 2A). The 21-day p.o. regimen with BWP (600 mg/kg) significantly suppressed neuronal cell damage (\( P<0.05 \), Fig. 2A). The 14 days pre-ischemic administration but not 1 day and 7 days pre-ischemic and 7 days post-ischemic administration prevented the cell damage in the hippocampal CA1 area (data not shown).

The TUNEL positive cell shown in hippocampal CA1 region were divided into 3 grades: severe, \( >50 \) TUNEL-positive cells/mm\textsuperscript{2}; mild, \( <25 \) TUNEL-positive cells/mm\textsuperscript{2}; normal, \( <10 \) TUNEL-positive cells/mm\textsuperscript{2}. Apoptotic cells were observed in eight of eight vehicle-treatment rats (\( >50 \) TUNEL-positive cells/mm\textsuperscript{2}). In the BWP-treatment group (600 mg/kg, 21-day), four of eight rats were completely inhibited (\(<10 \) TUNEL-positive cells/mm\textsuperscript{2}), three of eight rats were mild inhibited (\(<25 \) TUNEL-positive cells/mm\textsuperscript{2}), and one of
eight rats was severe (>50 TUNEL-positive cells/mm²). The 21-day p.o. regimen with BWP (600 mg/kg) attenuated apoptotic cell death in the hippocampal CA1 (Fig. 2B).

Effect of the 14-day p.o. regimen with BWP on excess glutamate release in the hippocampal CA1 area of rats subjected to repeated ischemia

BWP (600 mg/kg) was administered p.o. for 13 continuous days, and on the 14th day, BWP was administered 30 min before glutamate release was measured. As shown in Fig. 3, during the first occlusion, BWP tended to decrease excess glutamate release (vehicle treatment: 207.1 ± 36.8%, BWP treatment: 166.4 ± 47.3%). During the second occlusion, BWP significantly decreased the excess release of glutamate (vehicle treatment: 184.3 ± 29.6%, BWP treatment: 97.9 ± 10.9%, P<0.05) compared with the vehicle-treated group.

Effect of the 14-day p.o. regimen with BWP on the delayed increase in NO$_3^-$ levels in the hippocampal CA1 area of rats subjected to repeated ischemia

The 14-day p.o. regimen with BWP (600 mg/kg) did not affect basal NO$_3^-$ levels in the sham-treated group. As shown in Fig. 4, the vehicle-treated group of repeated ischemia slowly increased NO$_3^-$ levels in the hippocampal CA1 area from 90 min after the first occlusion. The 14-day p.o. regimen with BWP (600 mg/kg) significantly suppressed the delayed increase in NO$_3^-$ levels in the hippocampal CA1 area of the rats subjected to repeated ischemia. The repeated measures of two-way ANOVA revealed the effect of group (F(2,8) = 8.885, P<0.01), effect of time (F(16,128) = 2.892, P<0.001) and a group x time interaction F(32,128) = 4.153, P<0.001). Post-hoc analysis revealed that the NO$_3^-$ levels during 30 min and 80 min to 240 min after the first occlusion were lower in the BWP-treated group than in the vehicle-treated group (P<0.05, Tukey’s test).

Effect of BWP on hippocampal blood flow and blood pressure

As shown in Fig. 5A, both single and repeated treatment with BWP (600 mg/kg) did not affect hippocampal blood flow in normal rats. The 14-day regimen with BWP (600 mg/kg) did not significantly increase blood flow in the hippocampal region after occlusion (Fig. 5B). Both single and repeated treatment with BWP (600 mg/kg) also did not affect blood pressure in normal rats (normal: 107.2 ± 3.5 mmHg, BWP single treatment: 106.2 ± 5.3 mmHg, BWP 14-day treatment: 108.6 ± 4.7 mmHg).

Discussion

The present experiment demonstrated that the 21-day p.o. regimen with BWP (14-day pre-ischemic and 7-day post-ischemic administration with 600 mg/kg...
BWP) significantly prevented the impairment of spatial memory in the 8-arm radial maze test and decreased both necrosis and TUNEL-positive cells in the hippocampal CA1 area in rats subjected to repeated cerebral ischemia. On the other hand, 7-day post-ischemic administration with BWP failed to do so. In order to investigate the mechanism of BWP, glutamate release and NO\textsubscript{x}\textsuperscript{-} were measured using in vivo microdialysis. BWP inhibited excess glutamate release during the second occlusion and suppressed the delayed increase in NO\textsubscript{x}\textsuperscript{-} induced by repeated ischemia. However, BWP did not affect either hippocampal blood flow in either intact rats or rats subjected to repeated ischemia as determined by laser Doppler flowmeter or blood pressure in intact rats. These results suggest that pre-ischemic administration with BWP may produce neuroprotective action against repeated cerebral ischemia.

It is reported that 20-day pre-ischemic administration of BWP improved the activities of antioxidation enzyme, superoxide dismutase, catalase, and glutathione peroxidase in a renal ischemia-reperfusion model (20). However, behavioral and histological studies were performed 7 days after the occlusion, and biochemical studies (glutamate release and NO production) and

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**Fig. 2.** Effects of the 21-day p.o. regimen with BWP on repeated cerebral ischemia-induced cell death and TUNEL-positive cells in the hippocampal CA1 region. A: Surviving cells in the CA1 region of the hippocampus assessed by HE staining. The number of cells is represented per 1 mm\textsuperscript{2}. B: TUNEL-positive cells (green) in the CA1 of the hippocampus. a: sham group, b: vehicle treatment group, c: BWP (600 mg/kg) treatment group. Magnification × 400 (bar = 25 μm). **P<0.001 vs sham group, *P<0.05 vs vehicle group (Mann-Whitney U test). Number of rats, 5 – 15.
blood flow were measured immediately after the occlusion. This repeated ischemia model can be useful as an animal model for cerebrovascular dementia because the relation between spatial memory impairment and cell damage in the hippocampal CA1 area can be examined directly. For these reasons, we employed this experimental schedule. It is important to examine the therapeutic time-window of neuroprotective compounds in stroke. To investigate the time-window of BWP in the repeated ischemia model, we compared 14-day pre-ischemic administration with 7-day post-ischemic administration. The results demonstrated that 14-day pre-ischemic but not 7-day post-ischemic administration of BWP provides cerebral neuroprotection against repeated cerebral ischemia. On the other hand, we investigated the effect of BWP in a single treatment and a 7-day treatment on the neuronal injuries induced
by repeated cerebral ischemia, and they did not inhibit the neuron death in the region of CA1 hippocampus (data not shown). So we think that pre-ischemic 14-day treatment is necessary.

In the present experiment, BWP inhibited excess glutamate release not only the second occlusion but also tended to decrease the excess glutamate release during the first occlusion, but it was not significant by statistical analysis. We have reported that the single occlusion did not cause the spatial memory impairment and neuronal apoptosis at 7 days after ischemia (3), and the single occlusion did not produce a delayed increase in the NO$_x$ levels at 2 – 4 h after occlusion. The result suggests that the excess glutamate release during the second occlusion is more important for the spatial memory impairment and induction of apoptosis in the hippocampus in rats subjected to repeated ischemia than the single occlusion. These results suggest that the inhibitory effect of glutamate release during second occlusion may be explained as the neuroprotective action of BWP.

It is known that the pathway of glutamate release / AMPA receptor / Ca$^{2+}$ influx / NO production is important in the cerebral ischemia model (25). We have reported that nimodipine (4), YM-90K (3), or L-NAME (5), as mentioned, could ameliorate both the impairment of spatial memory in 8-arm radial maze and the neuronal damage induced by cerebral repeated ischemia. In the present study, glutamate release transiently increased immediately after both occlusions, and NO$_x$ produced a delayed increase after the second occlusion. The results suggest that the excess glutamate release and delayed increase of NO$_x$ are thought to be the triggers of neuronal degeneration in repeated cerebral ischemia.

As mentioned above, EGCG possesses a neuroprotective effect against AMPA-induced neuronal damage through inhibition of AMPA-induced intracellular Ca$^{2+}$ increase in hippocampal neurons (2). It was also reported that the AMPA receptor played a more important role than the NMDA receptor of glutamate in the incidence of neuronal damage in a cerebral ischemia model (26 – 28). It was regarded that the change in GluR2 could modulate the influx of Ca$^{2+}$ and was connected to the cell damage after cerebral ischemia reperfusion (29). Thus, these results suggested that the neuroprotective action of BWP might be related to the AMPA receptor. Therefore, we studied the effect of BWP on mRNA of GluR2, one subunit of the AMPA receptor, by RT-PCR. Fourteen-day BWP treatment didn’t affect the decrease of GluR2 mRNA at 24 h after repeated cerebral ischemia (unpublished data). On the other hand, 14-day BWP treatment could significantly inhibit excess glutamate release during the second occlusion. These results suggest that the neuroprotective action of BWP is not mediated through the AMPA receptor directly, but through inhibiting the presynaptic glutaminergic nerve indirectly.

The reactive oxygen radical induced by ischemia-reperfusion causes post-ischemic neuronal damage (30). It has been reported that antioxidation protects neurons from damage induced by ischemia. We also reported that the vitamin E isoforms α-tocotrienol and γ-tocopherol prevent cerebral infarction in mice (31). Resveratrol, quercetin, and catechin, three major red wine-derived polyphenols, protected against toxicity induced by NO free radical donors, like sodium nitroprusside (SNP), in cultured hippocampal neurons (7). Moreover, catechin and other green tea polyphenols showed protective effects through prevention of lipid peroxidation and a radical-scavenging effect (11). BWP showed higher activities of antioxidant enzymes as mentioned above (21). During cerebral ischemia-reperfusion, NO generated from neuronal nitric oxide synthases (nNOS) quickly reacts with superoxide anion induced by ischemia-reperfusion and forms peroxynitrite anion to cause cell damage (32). Therefore, we examined whether BWP could suppress the delayed increase in NO$_x$ induced by repeated ischemia in the dorsal hippocampus measured by in vivo microdialysis. The results demonstrated that BWP could significantly suppress the delayed increase in NO$_x$ induced by repeated ischemia. It has been determined that NO is generated by three isoforms of nitric oxide synthase; and in the cerebral ischemia model, the selective inhibition of nNOS and inducible nitric oxide synthase (iNOS) are neuroprotective, while selective inhibition of endothelial nitric oxide synthase (eNOS) is neurotoxic (33, 34). NO generated by eNOS acts as a vasodilator to increase cerebral blood flow in the ischemia region and decreases the injury induced by ischemia (35). The wine polyphenols possess potent endothelium-dependent vasorelaxing activity through enhancing NO synthesis in the rat aorta (36). Quercetin and its metabolites also possess vasodilator effects with selectivity toward the resistance vessel (37). Therefore, we examined the effect of BWP on hippocampal blood flow in intact rats and rats subjected to repeated ischemia and the effect on the blood pressure in intact rats. BWP did not affect the hippocampal blood flow or blood pressure in normal animals or the hippocampal blood flow in the repeated ischemic condition. This means that BWP does not affect the activity of eNOS. It was determined that NO generated from iNOS occurred at 24 h or more after cerebral ischemia (38). Taking these results together, it was suggested that BWP inhibited the acti-
vity of nNOS to suppress the NO\textsuperscript{−} level in the dorsal hippocampus in rats subjected to repeated ischemia and may act as free radical scavenger.

BWP contains various polyphenolic components such as catechin, epicatechin, quercetin, rutin, and hyperoside. The structures of these components that contain a 7,5 hydroxyl group in the A ring and a 3',4' hydroxyl group in the B ring benefit antioxidant activities. This component acts through the dissociation of a hydroxyl group to suppress the superoxide-driven Fenton Reaction, chelate transition metals, and change stable structure. On the other hand, catechin and epicatechin contained in tea were reported to scavenge intracellular active oxygen and act against ischemia reperfusion induced neuronal death in the gerbil (39). Quercetin in red wine showed antioxidation and protective action in cultured hippocampal neurons (20). Rutin was reported to act as an efficient radical inhibitor and have a neuroprotective effect against ischemia and reperfusion-induced cerebral injury (40), and could be metabolized to quercetin by oral administration (41). Hyperoside has anti-inflammatory effects and enhances the antioxidative capacity (17, 18). Thus, some components of BWP possess protective effects. Although it remains unclear which active component of BWP shows neuroprotective effects on repeated cerebral ischemia, the neuroprotective abilities of BWP may be a collaboration of multicomponents in BWP. On the other hand, it has been reported that the antioxidant capacity of fractions of catechin is related to the number of catechin units. The monomer catechin scavenges free radical by iron chelation, and the longer chain oligomer protects lipids from oxidation. Eighty percent of the BWP mixture is an oligomer of catechin and epicatechin. Thus it also suggested that the action of the oligomer of catechin and epicatechin might also play a role against impairment of spatial memory and the incidence of cell damage induced by repeated ischemia.

In conclusion, BWP has a neuroprotective effect on repeated cerebral ischemia in rats. The neuroprotective action of BWP may be mediated through the following mechanism: 1) BWP inhibits excess glutamate release induced by repeated cerebral ischemia; 2) BWP may act as a free radical scavenger, especially for NO; 3) BWP may improve the activity of antioxidation enzymes to scavenge intracellular oxygen radicals. BWP exerted the neuroprotective effect against hippocampal cell death, although it is not clear whether these effects are direct. We are interested in confirming the active compound of BWP that prevented neuronal necrosis and apoptosis. These results suggest that BWP may be useful for treating cerebrovascular disease.

References


