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ORIGINAL CONTRIBUTION

Dai-Eun Sok
Sang Hee Oh
Yun-Bae Kim
Hyun-Gu Kang
Mee Ree Kim

Neuroprotection by extract of *Petasites japonicus* leaves, a traditional vegetable, against oxidative stress in brain of mice challenged with kainic acid

Summary *Background* Reactive oxygen radicals have been implicated in the pathophysiology of many neurologic disorders and brain dysfunctions. Kainic acid has been used as a model agent for the study of neurotoxicity of various excitatory amino acids, since it induces neuronal damage through excessive production of reactive oxygen species. *Petasites japonicus* MAX (butterbur), cultivated as culinary vegetables in Eastern Asia, contains various kinds of phenolic compounds as well as sesquiterpenes, such as petasin. In European countries, the extracts from roots of *Petasites* species have been used in the therapy of headache or asthma. *Aim of the study* The objective of our study is to examine the neuroprotective action of the *Petasites japonicus* MAX (butterbur) extract against oxidative damage in the brain of mice treated with kainic acid. *Methods* Male ICR mice, 6–8 weeks of age, were administered orally the butanol fraction from methanol extract of *Petasites japonicus* (BMP) or its subfraction (BMP-I or BMP-II) for 5 consecutive days. Thirty min after the final administration, the animals were challenged s. c. with kainic acid (45 mg/kg), and neurobehavioral activities were monitored. In addition, biomarkers of oxidative stress and neuronal loss in the hippocampus for the biochemical, neurobehavioral, morphological evaluations were analyzed 2 days after the kainic acid challenge. *Results* During 5-day treatment with BMP or BMP-1, the body weight gain was not significantly different from that of vehicle-treated control animals. Administration of kainic acid alone induced severe epileptiform seizures, causing a lethality of approximately 50 %, and injuries of pyramidal cells in the hippocampus of mice which survived the challenge. Kainic acid exposure also resulted in a remarkable decrease in total glutathione level and glutathione peroxidase activity, and an increase in the thiobarbituric acid-reactive substance (TBARS) value in brain tissues. In comparison, coadministration with BMP (400 mg/kg) reduced the 54 % lethality of mice, administered with kainic acid alone, to 25 % ($P < 0.05$). Moreover, BMP at the same dose restored the levels of reduced glutathione and TBARS to control values ($P < 0.05$). In further studies, BMP-I (200 mg/kg) ameliorated significantly ($P < 0.05$) the kainic acid-induced behavioral signs, such as seizure activity, and all mice administered with BMP-I (200 mg/kg) survived the kainic acid toxicity. Consistent with the above, the administration with BMP-1 remarkably attenuated the neurobehavioral signs and neuronal loss in hippocampal CA1 and CA3 regions. *Conclusion* On the basis of these results, the butanol fraction, especially BMP-I, of *Petasites japonicus* MAX extract is possibly suggested to be a functional agent to prevent oxidative damage in the brain of mice.

Key words *Petasites japonicus*-kainic acid – neuroprotection – glutathione – lipid peroxidation

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D.-E. Sok
College of Pharmacy
Chungnam National University
Daejeon, Korea

S. H. Oh · Prof. M. R. Kim, Ph.D. (✉)
Dept. of Food and Nutrition
Chungnam National University
220 Gung-Dong, Yuseong-Gu
Daejeon 305-764, Korea
Tel.: +82-42/821-6837
Fax: +82-42/822-8283
E-Mail: mrkim@cnu.ac.kr

Y.-B. Kim · H.-G. Kang
College of Veterinary Science and Research
Institute of Veterinary Medicine
Chungbuk National University
Cheongju, Korea

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Abbreviations

BMP	butanol fraction of methanol extract from *Petasites japonicus* MAX
GSH	reduced glutathione
KA	kainic acid
TBARS	thiobarbituric acid-reactive substances
DTNB	5.5'-dithiobis (nitrobenzoate)

Introduction

Kainic acid (KA) is a potent central nervous system excitotoxin producing acute and subacute epileptiform activity, ultimately resulting in widespread irreversible neuropathological change [1]. KA binds to and activates a subtype of ionotropic glutamate receptors [2]. Besides inducing brain lesions directly, KA can provoke the release of potentially neurotoxic amounts of glutamate [3]. Therefore, kainic acid (KA) has been used as a model agent for the study of neurotoxicity of various excitatory amino acids such as glutamate. KA-induced neuronal death may result from the generation of reactive oxygen species (ROS) and subsequent membrane destruction, consistent with the ability of lipophilic antioxidants to reduce KA neurotoxicity [4, 5]. The brain may be particularly vulnerable to oxidative stress in that it consumes a large amount of the body oxygen while having a relative paucity of protective systems [3, 6]. Glutathione, a major antioxidant in tissue defense against oxidative stress in tissues including brain, participates enzymatically and non-enzymatically in maintaining cellular redox balance and in protecting against reactive oxygen species (ROS)-mediated oxidative damage [6–8]. The intracellular level of reduced glutathione is maintained mainly by glutathione reductase. Additionally, the enzymes such as superoxide dismutase, catalase, or glutathione peroxidase also contribute to the preservation of intracellular GSH level [9] by clearing ROS or preventing ROS formation. Consistent with this, the administration of melatonin or GSH prevented kainic acid neurotoxicity through their antioxidant action in brain tissue [4, 5]. In this regard, the plant antioxidants, which can penetrate the blood brain barrier, are expected to express a good neuroprotective action [10]. Previously, the extract from *Aster scaber*, which showed a strong antioxidant activity in PC12 cells [11], was found to exert a neuroprotective action in mice intoxicated with NMDA [12]. Likewise, it is expected that a neuroprotective action may be expressed by the extract from *Petasites japonicus* MAX, which is known to contain antioxidant polyphenols [13, 14], and compounds capable of blocking Ca^{2}+ channel [15, 16]. *Petasites japonicus* MAX is cultivated as culinary vegetables in Eastern Asia including Korea, Japan, and Taiwan. In European countries, the extract from roots of *Petasites* species has been in therapeutic use [15]. The refined preparation (Ze 339) of ethyl acetate extract of *Petasites hybridus* root has been used in prophylactic treatment of migraines or gastric ulcers, and as an antispasmodic agent for asthma [15, 17]. The main bioactive constituents in the ethyl acetate extract of Petasites species root are sesquiterpenes, such as petasin or isopetasin. A disadvantage with the extract of the plant root is that it contains a high level of toxic pyrrolizidine alkaloids [15, 18]. Meanwhile, the leaves of *Petasites* species, containing a negligible level of pyrrolizidine [15], have been reported to contain antioxidant components, relatively polar, such as flavonoid glycoside [14], fukinolic acid [19] or petasiformin A [20]. Nevertheless, the neuroprotective action of the extract from Petasites leaves has not been investigated.

The objective of our study is to examine the neuroprotective action of the butanol fraction from *Petasites japonicus* MAX extract against oxidative damage in the brain of mice treated with kainic acid. For this purpose, the neurobehavioral, biochemical, and morphological changes were evaluated in mice challenged with kainic acid.

Materials and methods

Materials

Kainic acid, glutathione reductase (Type III from bakers yeast), reduced glutathione (GSH), oxidized glutathione (GSSG), tetramethoxypropane, 5,5'-dithio-2-nitrobenzoic acid (DTNB), NADP, NADPH, thiobarbituric acid (TBA) and bovine serum albumin were products of Sigma Chemical Co. (St. Louis, MO). *S*-Petasin was kindly provided by Dr Chieh-Fu Chen, National Research Institute of Chinese Medicine, Taipei, Taiwan, ROC.

Plant source

The leaves of *Petasites japonicus* were collected from agricultural fields in Yangchon, Korea in May, 2004, and verified by Dr. Young Jin Choi. A voucher specimen was deposited in the Herbarium of Wild Vegetable Experiment Station, Kangwon-Do, Korea.

Preparation of vegetable extracts

Washed and chopped fresh *Petasites japonicus* MAX was dried in a freeze dryer (at –70 °C). The dried leaves (1.4 kg) of *Petasites japonicus* MAX were extracted three times with methanol in the dark (15 °C). Methanol extract (310 g), after concentration, was suspended in distilled water, and the suspension was partitioned three

times with hexane, chloroform, ethyl acetate and n-butanol, successively (Fig. 1). Each fraction was evaporated at low temperature under reduced pressure, and then used for in vitro or in vivo experiments. Separately, the butanol fraction (BMP) was dried and then resuspended in 10 volumes of n-butanol. After the centrifugation (17,000 g, 10 min) of the mixture, the supernatant and the butanol-insoluble pellet were dried under vacuum evaporation to give BMP-I and BMP-II, respectively.

Prevention against lipid peroxidation in vitro

Metal-mediated lipid peroxidation in brain homogenate was induced with Fe^{+2} and ascorbic acid *in vitro*. Brain homogenate was incubated with 0.2 mM Fe^{+2} and 25 mM ascorbic acid in the presence or absence of vegetable extract, and the mixture was placed in a shaking water bath at 37 °C. After 30 min, equal volumes of 15% trichloroacetic acid (TCA) and 0.75 % thiobarbituric acid (TBA) were added to the mixture. The reaction mixtures were heated in boiling water for 15 min, kept in ice for 5 min, and then centrifuged (3,000 rpm, 10 min) to separate corpusculate particles. The absorbance (A) of the supernatant was measured using a spectrophotometer at 533 nm. Calibration was performed using a malondialdehyde standard prepared by hydrolysis of 1,1,3,3,-tetraethoxypropane [12]. IC_{50} values for the inhibition of lipid peroxidation were determined as described previously [21].

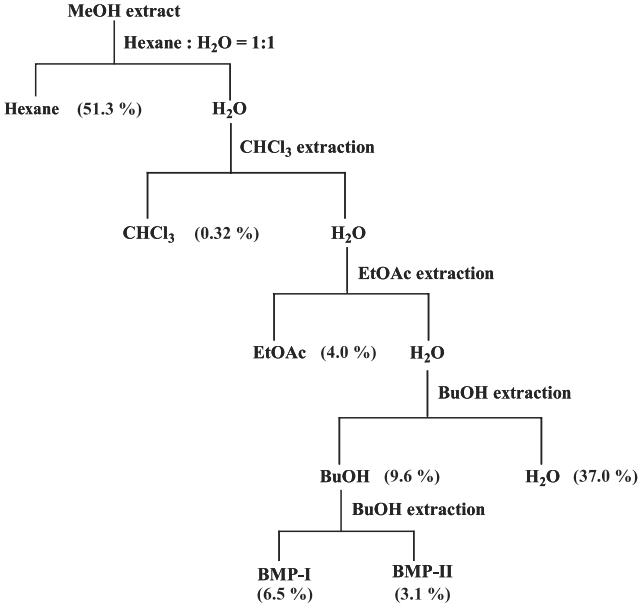

Fig. 1 Systematic solvent fractionation of *Petasites japonicus* MAX

Animal experiments

Male ICR mice (25–30 g), 6 to 8 weeks of age, were housed in polycarbonate cages (8 animals per cage, identified using the ear punch method), and fed unrestricted amounts of filtered water and pelleted commercial diet (Samyang Co., Korea). The temperature and relative humidity were 23 ± 3 °C and 55 ± 10 %, respectively, and 12-h light/12-h dark cycles were maintained. All animal experiments were conducted in compliance with 'Guide for Care and Use of Laboratory Animals' of the National Institutes of Health Guidelines [22]. The mice, after one week acclimation to the laboratory environments, were assigned randomly to treatment groups (13–20 mice/group) and were weighed individually every day. Mice were administered orally with the butanol fraction (200 or 400 mg/kg, 5 ml/kg) of *Petasites* leaves, suspended in saline, or saline as vehicle using a gavage needle for 5 consecutive days before kainic acid injection. Thirty minutes after the final administration, the animals were challenged by s. c. injection with 45 mg/kg (3 ml/kg) of kainic acid (10 mM phosphate-buffered saline, pH 7.4), a dose which had been previously confirmed to induce seizure-related brain injuries [21, 23].

Following the challenge with kainic acid, the onset time of neurobehavioral activities such as tail arch, tremors or seizures, and the mortality of the animals were monitored for 2 h [24–27]. Most of the animals exhibited tail arch, and finally reached tremors, followed by seizures or death. In addition, such behavioral activities were intermittently recurrent, rather than persistent. So, we recorded the incidence and onset time of first sign, rather than measuring the intensity or duration of symptoms. Two days after the kainic acid administration, the brain tissues of mice were removed after intracardial perfusion, under light anesthesia with ether, with cold saline to avoid blood contamination. Out of 13–15 mice (each group), which survived the kainic acid challenge, 8 mice were subjected to biochemical and morphological assessments, and the remaining 5–7 mice to statistical analysis of neuronal loss. For biochemical and morphological assessments, the left hemisphere of the brain was frozen in liquid nitrogen, and stored at –80 °C until used (within 48 h) for the biochemical and enzymatic analyses. The other part (right hemisphere) of the brain was fixed in 10 % neutral formalin solution for morphological findings of neurotoxicity.

Measurement of lipid peroxidation by thiobarbituric acid reacting substances (TBARS)

Brain tissue, rinsed with 0.15 M KCl solution containing 2 mM EDTA, was homogenized in nine volumes of 10

mM phosphate buffer (pH 7.4) using a tissue homogenizer with a Teflon pestle. To the brain homogenate (1.0 ml) was added 1.0 ml of 8.1 % SDS, 2 ml of 20 % acetic acid and 1 ml of 0.75 % TBA. The mixture was boiled for 30 min, then centrifuged (14,000 rpm, 10 min), and then the absorbance of the supernatant was measured at 533 nm as previously described [28].

Determination of total GSH

Brain tissue (about 0.2 g wet wt) was pulverized in a cooled ceramic percussion mortar with 6 % metaphosphoric acid, and the mixture was centrifuged (27,000 × g, 20 min) at 4 °C. Total GSH was determined enzymatically according to a published procedure [29, 30] with a slight modification. To 0.05 ml of the supernatant was added 0.39 mL of 100 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.025 ml of 10 mM DTNB and 0.08 ml of 5 mM NADPH. After 3 min equilibration at 25 °C, the reaction was started by adding 2 units of GSH reductase. The formation of 2-nitro-5-thiobenzoic acid was continuously recorded at 412 nm with a UV/VIS spectrophotometer. The total amount of GSH in the samples was determined from a standard curve obtained by plotting the known amount of GSH vs. the rate of change of absorbance at 412 nm.

Morphological examination

For the microscopic evaluation, a part of the brain was fixed in 10 % neutral formalin solution, and the formalin-fixed brain tissues were processed and embedded in paraffin. Serial coronal sections (4 μm in thickness), including dorsal hippocampus, were obtained, and stained with hematoxylin and eosin. The histopathological findings of limbic system including hippocampus were routinely assessed under a light microscope. For statistical analysis of the degree of neuronal loss in hippocampus, the tissue sections of both hemispheres from 5–7 animals/group were also stained with hematoxylin and eosin, and the surviving pyramidal neurons in CA1 (per 500 μm) and CA3 (under 200-fold magnification) regions were counted bilaterally and averaged.

Protein determination

Protein was determined according to the method of Bradford [31] using bovine serum albumin as a standard.

Statistical analyses

All statistical analyses were performed using a SAS program [32]. Duncan's multiple range test was used to determine a significant difference among treatment groups after initial demonstration of a treatment-related effect by analysis of variance. All data are presented as mean ± standard error (S. E.). Statistical assessments were performed using ANOVA, followed by post-hoc Duncan's multiple-range test for biochemical and neurobehavioral changes or Scheffe's test for histopathology [33]. Statistical significance refers to results where $P < 0.05$ was obtained.

Results

In vitro screening of antioxidant fractions from Petasites japonicus MAX extract

To select a fraction of *Petasites japonicus* MAX extract expressing a potent antioxidant action, each fraction (Fig. 1) was examined for the capacity to prevent brain membrane from lipid peroxidation. The greatest protection against lipid peroxidation of brain membrane was shown by ethyl acetate or butanol fraction with an IC_{50} value of 20–23 μg/ml. Since the ethylacetate fraction had been reported to contain toxic pyrrolizidine alkaloid [15], the butanol fraction (BMP) of *Petasites japonicus* MAX extract was employed in the next study to evaluate the neuroprotective action *in vivo*.

Effect of butanol fraction (BMP) on the behavioral change and lethality

First, the neurotoxic effect of kainic acid on the behavior of male mice was investigated. In repeated experiments, there was a narrow margin between doses to trigger seizures and to cause death in mice as had been observed previously [34]. Therefore, the dose of kainic acid was chosen to induce seizure-related brain injuries. A single administration of kainic acid (45 mg/kg) caused a typical sustained seizure, which was evident 10 min after s. c. administration, with a tremor persisting for 20–30 min, in contrast to the vehicle-treated group, which showed no seizure activity. In the following experiment, where the butanol fraction (BMP) at a dose of 400 mg/kg was administered by gavage to mice before the exposure to kainic acid, the mortality in BMP/kainic acid-treated mice group decreased to 25 %, in contrast to 54 % in vehicle/kainic acid-treated group. However, a lower dose (150 mg/kg) of BMP failed to significantly reduce the lethality. Separately, the administration with BMP (400 mg/kg) caused no significant change in body or brain weight.

Effect of BMP on lipid peroxidation in the brain of kainic acid-treated mice

To see whether the neuroprotective action of BMP was related to the prevention against oxidative stress in the brain tissue of mice intoxicated with kainic acid, we examined the effect of BMP on the level of TBARS, a biochemical marker of oxidative stress, in the brain of mice administered with kainic acid (45 mg/kg). Fig. 2A shows that TBARS value was increased to 122% of control value ($P < 0.05$) in the homogenate of whole brain of mice treated with kainic acid. Meanwhile, BMP at a dose of 400 mg/kg reduced the TBARS value, which was enhanced by kainic acid challenge, to the level of control group ($P < 0.05$). These results suggest that the neuroprotective action of BMP may be in part due to its preventive action against oxidative stress in the brain.

Effect of BMP on glutathione level in the brain of kainic acid-treated mice

Next, we examined the effect of BMP on the level of total glutathione, another biomarker of oxidative stress, in the brain of mice administered with kainic acid (45 mg/kg). As shown in Fig. 2B, the administration of kainic acid (45 mg/kg) reduced the level of total glutathione in the cytosol fraction of brain homogenate to approximately 73% of control level ($P < 0.05$). Then, the administration of BMP (400 mg/kg) elevated the level of total GSH in the brain cytosol from 73% in kainic acid-treated group to 105% of control group ($P < 0.05$), further confirming the notion that the neuroprotective effect of BMP may be related to its prevention against oxidative stress in the brain.

Effect of BMP-I or BMP-II on behavioral change and mortality

After having established the neuroprotective action of BMP, we further evaluated the neuroprotective effect of two subfractions from BMP, BMP-I or BMP-II. When the butanol fraction (BMP-I) at a dose of 200 mg/kg was administered before the exposure to kainic acid, the onset time of neurobehavioral change, such as tailoring, tremor or convulsion, was significantly ($P < 0.05$) delayed in the BMP-I/kainic acid-treated mice as compared to the vehicle/kainic acid-treated mice (Table 1). Moreover, all of mice pretreated with BMP-I survived the kainic acid challenge, in contrast to 54.5% of mortality in the group treated with kainic acid alone. In contrast, BMP-II had no significant effect on the behavioral change and mortality. Thus, BMP-I is suggested to contain anticonvulsant compounds, which show a neuroprotective action. In a separate experiment, the administration of BMP-I (200 mg/kg) just after kainic acid injection failed to significantly prevent kainic acid toxicity, suggesting the candidate antioxidant compounds in BMP-I may require metabolism to exhibit an antioxidant action in brain tissue.

In addition, when the effect of BMP I (200 mg/kg) on

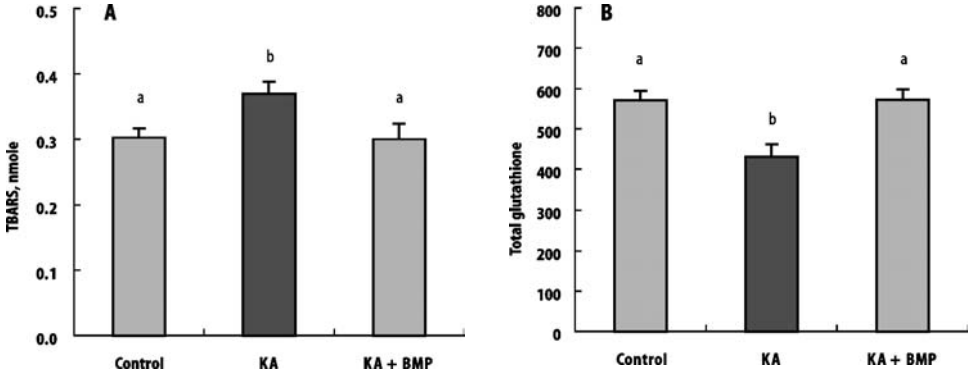

Fig. 2 Effect of BMP on biomarker levels in the brain tissues of mice (n = 8) administered with kainic acid. **A** Change in total GSH level. **B** Change in TBARS value. Values with the same letter are not significantly different ($P < 0.05$). Data are means ± S. E. of three determinations from six to eight mice

Table 1 Effect of BMP-I and BMP-II on the onset time (min) and incidence (%) of kainic acid-induced neurobehavioral changes and lethality of mice (n = 8). Values are means ± S. E. Any two means in the same row with different letters represent a significant difference at $P < 0.05$

Treatment (mg/kg)	Tail arch (%)	Tremors (%)	Seizures (%)	Mortality (%)
Vehicle + KA (45)	12.4±4.7[a] (100.0)	16.7±6.1[a] (100.0)	18.9±7.0[a] (100.0)	41.9±19.5[a] (54.5)
BMP-I (200) + KA (45)	27.6±6.4[b] (100.0)	40.6±11.9[b] (77.8)	48.2±10.4[b] (66.7)	– (0.0)
BMP-II (200) + KA (45)	11.4±7.6[a] (100.0)	17.0±9.7[a] (100.0)	22.6±16.8[a] (88.9)	43.8±21.5[a] (44.4)

body weight and brain weight was examined, it was found that BMP-I caused no significant change in body or brain weight (Table 2). Thus, mice survived the 5 day-gavage administration of BMP-1. Independently, BMP-1 was subjected to a simple adsorption process to further remove non-polar compounds. Then, when this refined BMP-1 preparation at a dose of 50 mg/kg was administered to mice, it was observed that the mortality (73 %) in kainite-treated group was decreased to 37 % of control. In addition, the behavioral sign such as tail arch was extended from 11 min to 22.3 min. These data suggest that the effective dose of BMP-1 may be reduced to 50 mg/kg.

Effect of BMP-I and BMP-II on hippocampal CA1 and CA2 regions

Kainic acid-induced generalized seizures are known to cause extensive injuries of pyramidal cells in hippocampal CA1 and CA3 regions. Consistent with the above, morphological changes in hippocampus were caused by kainic acid as demonstrated in Fig. 3. The injured cells exhibited dark degeneration and shrinkage, leading to a pericellular halo and spongiform change of neuropils. On the second day following the kainic acid exposure, only 18 % and 9 % of the neurons survived in CA1 and CA3 regions, respectively, indicative of excessive oxidative stress in brain tissues (Fig. 4). Meanwhile, such a loss of hippocampal neurons was greatly reduced in mice pretreated with BMP-I (200 mg/kg), in parallel with the protection against the seizure activity. In contrast, BMP-II (200 mg/kg) did not exert a significant protective effect on neuronal injuries. Thus, BMP-I appears to contain compounds, which attenuate the kainic acid-induced neuronal injuries.

Discussion

Some aspects of kainic acid-triggered excitotoxicity in the brain involve the production of reactive oxygen radicals, which are known to cause the reduction in GSH level, and the increase of lipid peroxidation in brain tissue [4, 35]. Consistent with this, the present study confirms that total GSH level and the TBARS value are biochemical indicators of kainic acid-induced oxidative imbalance in brain tissue of mice. Although the brain has endogenous antioxidants such as glutathione and melatonin, the ability of these antioxidants to combat oxidative stress in brain tissue is limited. Probably in support of this, the administration of glutathione or melatonin was observed to protect against kainic acid-induced neuropathological changes in rat brain [4, 5, 10], suggesting the value of a pharmacological strategy directed toward the regulation of the reducing power in brain tissue. To augment intracellular antioxidant capacity, the use of membrane-permeable antioxidants could be a potential approach.

In the present study, the administration of BMP, a butanol fraction of *Petasites japonicus* MAX extract, to mice treated with kainic acid exhibited a GSH-sparing activity as well as a preventive action against lipid peroxidation in mice brain. The ability of BMP to ameliorate behavioral signs of kainic acid neurotoxicity, and to reduce the lethality might be related to its prevention against oxidative stress. From these, it is assumed that the primary neuroprotective effect of BMP in vivo may be partly due to its antioxidant activity. It is noteworthy that the butanol fraction of *Petasites japonicus* MAX is more neuroprotective than the other edible plant extracts tested; in particular, the extract of *Petasites japonicus* leaves was more neuroprotective than that of *Aster scaber* leaves [21].

Generally, the antioxidant compounds are known to distribute in the butanol fraction or/and ethyl acetate fraction of plant extract. Previous studies indicated that the ethyl acetate fraction from *Petasites* species extract contained various bioactive sesquiterpene derivatives, such as petasin, isopetasin or S-petasin [16, 17, 36]. Petasin is responsible for the anti-spasmodic anti-inflammatory actions of the plant [36], which is ascribed to the blocking of Ca^{2+} channel, or the inhibition of leukotriene or prostaglandin synthesis. S-Petasin, another component, exerts a hypotensive action by blocking calcium channel in vascular smooth muscle [16]. In addition, petasiphenol, a polyphenol in the ethyl acetate fraction, was found to show radical-scavenging activity [37]. Despite its various bioactivities, the ethyl acetate fraction of *Petasites japonicus* was not successful to reduce the lethality caused by kainic acid alone. Even the intraperitoneal administration of *S*-petasin (24 mg/kg),

Table 2 Change of body weight and brain weight of mice (n = 8) administered with kainic acid and BMP. Values are means ± S. E. of determinations from four to eight mice. *N.S.* not significant at $P < 0.05$

Treatment	Initial body weight (g)	Final body weight (g)	ΔBody weight (g)	Brain weight (g)
Control	30.04±0.71$^{N.S.}$	30.09±0.95$^{N.S.}$	0.05±0.51$^{N.S.}$	0.304±0.0059$^{N.S.}$
KA (45 mg/kg)	29.49±0.91	29.88±0.86	0.39±0.29	0.327±0.0098
KA (45 mg/kg) + BMP-I (200 mg/kg)	31.66±0.65	31.49±0.74	0.18±0.39	0.328±0.0178
KA (45 mg/kg) + BMP-II (200 mg/kg)	30.56±0.88	29.33±1.16	1.12±1.16	0.319±0.0051

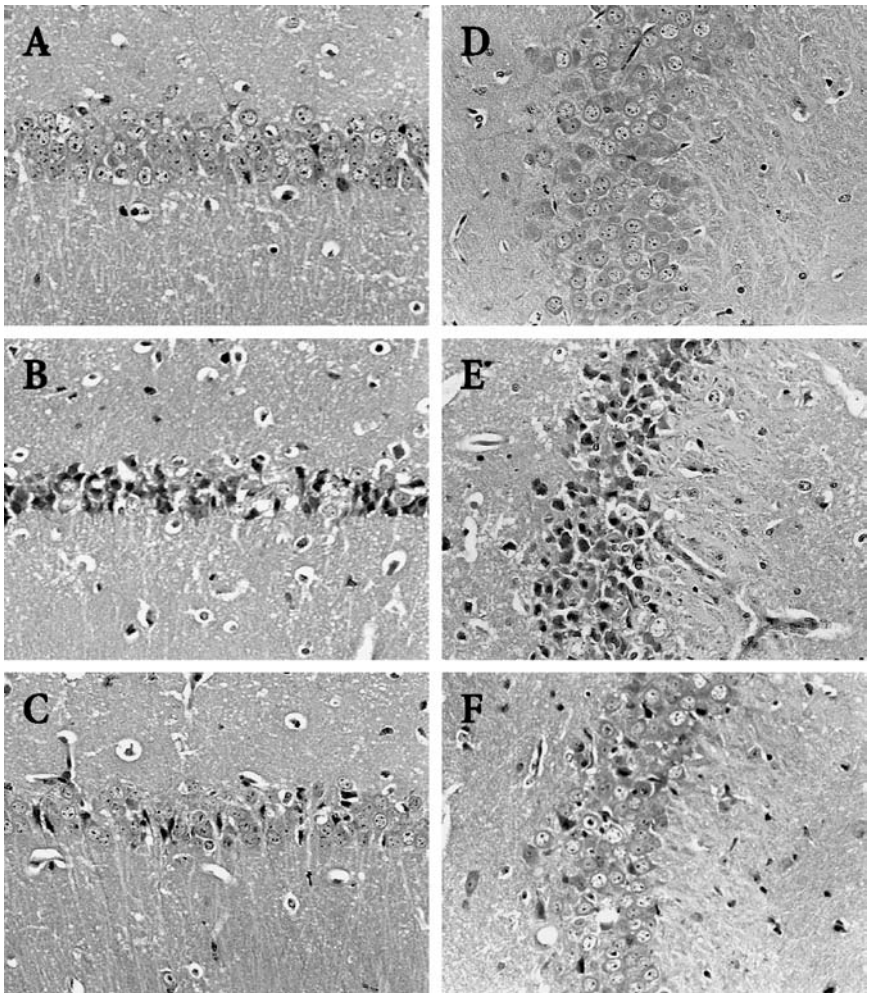

Fig. 3 Effect of BMP-I on kainic acid-induced neurotoxicity in hippocampal CA1 (**A–C**) and CA3 (**D–F**) regions. The injured cells exhibit dark degeneration and shrinkage, leading to a pericellular halo and spongiform change of neurophils. Such degenerate neurons were markedly increased in CA1 (**B**) and CA3 (**E**) regions in mice treated with kainic acid alone, in contrast to a few dead cells in normal mice (**A & D**). In comparison, a significant reduction in the number of degenerate neurons was observed in CA1 (**C**) and CA3 (**F**) regions in mice administered with BMP-I (200 mg/kg) and kainic acid

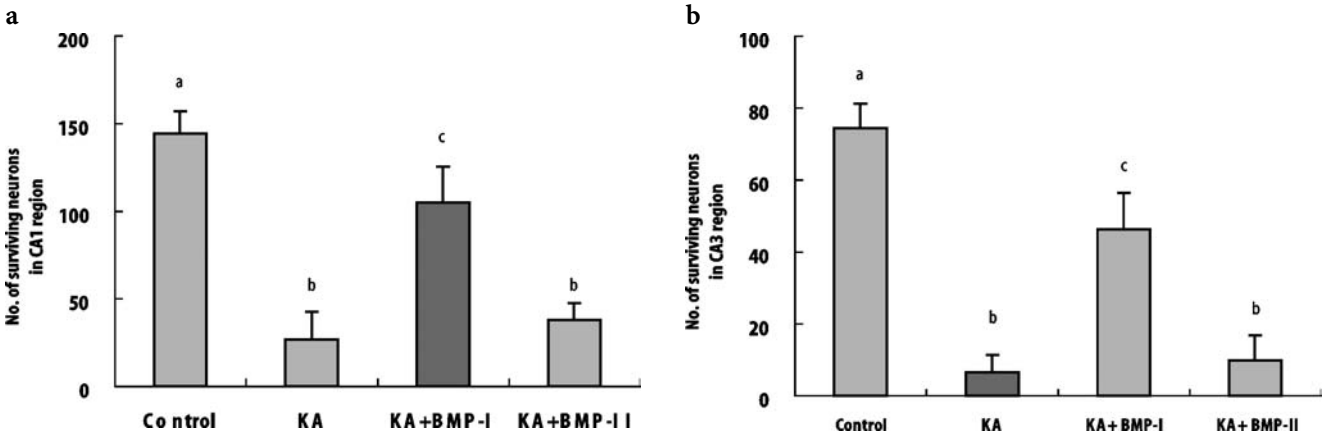

Fig. 4 Effect of BMP-I on the number of surviving neurons in hippocampal CA1 (**a**) and CA3 (**b**) regions. A significant recovery in the number of surviving cells was observed in mice administered with BMP-I (200 mg/kg) and kainic acid, in comparison with the cell number in mice treated with kainic acid alone. Data are means ± S. E. from both hemispheres of 5–7 mice. Values with the same letter are not significantly different ($P < 0.05$)

a sesquiterpenes, failed to reduce the lethality caused by kainic acid, suggesting no effect of *S*-petasin on the kainic acid neurotoxicity (unpublished data). Thus, the constituents present in the ethyl acetate fractions of *Petasites* species may not be responsible for the neuroprotective action. Generally, the butanol extract of plants is known to be rich in polyphenols. Recently, caffeinyl quinic acids, polyphenols from the butanol fraction of *Aster scaber* Thunb extract, were found to exhibit a neuroprotective effect in the PC12 cells exposed to kainic acid [11]. Very recently [38], some flavonoids such as patuletin, isolated from the butanol fraction of *Inula britannica*, were observed to prevent against oxidative stress in glutamate-injured cortical cells. Our recent data [21] indicate that the butanol fraction of *Aster scaber* Thunb extract shows a protective action against the excitotoxicity of kainic acid by exerting antioxidant action in the mice brain. Likewise, it is likely that the neuroprotective action of BMP-1 may be ascribed to polyphenols. According to earlier reports [13, 14, 19, 20, 37], the extract of Petasites species contains various polyphenols such as caffeic acid, fukinolic acid, petasiformin A, kaempferol 3-0-(6'-acetyl)-β-glucopyranoside or quercetin 3-0-(6'-acetyl)-β-glucopyranoside. Since the butanol fraction is considered to contain various polyphenols, the neuroprotective action of BMP-1 might be due to a combination of polyphenols rather than a single component. The higher neuroprotective action of BMP-I, compared to BMP-II, might be explained by the assumption that BMP-1 may contain a higher level of antioxidants that are blood brain barrier-permeable. However, it is not excluded that the active components must undergo metabolism to be neuroprotective. Accordingly, we administered the BMP or its subfraction prior to kainic acid challenge, so that the blood and brain concentrations of active ingredient(s) might be sufficient for the prevention of neuronal excitation or ensuing biochemical changes.

A key finding of the present study is that the butanol fraction from methanol extract of *Petasites japonicus*, an edible plant in Korea and Japan, may contain CNS-selective antioxidants to show a remarkable neuroprotective action against kainic acid excitotoxicity. Thus, the butanol fraction of *Petasites* species extract, especially BMP-I, might be useful in preventing neurodegenerative disorders caused by oxidative stress. Such an action of BMP-I as a neuroprotective agent may add to the usefulness of *Petasites species* extract in addition to the use of the ethylacetate fraction (Ze 339) of *Petasites hybridus* as an antimigraine [15] or antiasthma agent [17].

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