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Protection by Petaslignolide A, a Major Neuroprotective Compound in the Butanol Extract of *Petasites japonicus* Leaves, against Oxidative Damage in the Brains of Mice Challenged with Kainic Acid

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The neuroprotective effect of petaslignolide A (PA), a furfuran lignan isolated from butanol fraction of *Petasites japonicus* (Sieb. et Zucc.) Maxim. (Compositae) leaves, on the oxidative damage in the brain of mice challenged with kainic acid was examined using behavioral signs and biochemical parameters of oxidative stress. PA (40 mg/kg) was administered to ICR male mice through a gavage for 4 days consecutively, and on the final day, kainic acid (50 mg/kg) was administered intraperitoneally. During the 4-day treatment with PA, the body weight gain was not significantly different from that of vehicle-treated control animals. PA (40 mg/kg) alleviated the behavioral signs of kainic acid neurotoxicity and reduced the mortality (50%) by kainic acid to 12.5%. Moreover, the administration of PA restored the levels of glutathione and thiobarbituric acid-reactive substances as well as GSH-peroxidase activity in the brains of mice administered kainic acid to control levels ($P < 0.05$). In comparison, PA (40 mg/kg) was approximately comparable to the butanol fraction (200 mg/kg) of *P. japonicus* extract in reducing kainic acid neurotoxicity. On the basis of these results, PA is suggested to be a major neuroprotective agent primarily responsible for the protective action of the butanol fraction of *P. japonicus* extract against kainic acid-induced neurotoxicity in the brains of mice.

KEYWORDS: ***Petasites japonicus*; petaslignolide A; kainic acid; neuroprotection; glutathione; lipid peroxidation**

INTRODUCTION

Kainic acid (KA) is a potent central nervous system excitotoxin producing acute and subacute epileptiform activity, ultimately resulting in a widespread irreversible neuropathological change (*1*). Besides inducing brain lesions directly by activating a subtype of ionotropic glutamate receptors (*2*), KA can provoke the release of potentially neurotoxic amounts of glutamate (*3*). Therefore, 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, which can be prevented by some lipophilic antioxidants (*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 the brain, participates enzymatically and nonenzymatically in maintaining cellular redox balance and in protecting against ROS-mediated oxidative damage (*6*−*8*). The intracellular level of reduced glutathione is maintained mainly by glutathione reductase (*7*). Additionally, enzymes such as superoxide dismutase, catalase, or glutathione peroxidase also contribute to the preservation of intracellular reduced gluathione (GSH) level (*9*) by clearing ROS or preventing ROS formation. Consistent with the above, the administration of GSH in the brain prevented kainic acid neurotoxicity through its antioxidant action in brain tissue (*4*). In addition, melatonin or *trans*-resveratrol, membrane-permeable, showed the neuroprotective action against KA-induced neurotoxicity (*5*, *10*). In this regard, plant antioxidants, which can penetrate the blood−brain barrier, are expected to express a good neuroprotective action by removing ROS or preventing ROS formation (*11*). Recently, the butanol fraction from *Aster scaber*, an edible plant, was found to express a strong antioxidant activity in PC12 cells exposed to kainic acid (*12*), and moreover, exert a significant neuroprotective action in mice intoxicated with KA (*13*). Very recently, the butanol fraction from *Petasites japonicus* leaves, another edible plant, expressed a remarkable neuroprotective action against KA neurotoxicity (*14*). *P. japonicus* Maxim. is cultivated as a culinary vegetable in eastern Asia

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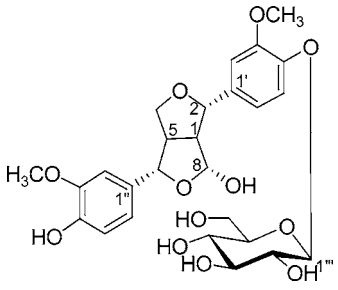

Figure 1. Structure of petaslignolide A.

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 the ethyl acetate extract of the *Petasites hybridus* root has been used in prophylactic treatment of migraines, gastric ulcers, and asthma (*15*, *16*). In addition, the extract of *Petasites formosanus* contains *S*-petasin, a hypotensive sesquiterpene from *P. formosanus* (*17*). However, a disadvantage with the crude ethyl acetate extract of *Petasites* species root is that it contains a high level of toxic pyrrolizidine alkaloids (*15*, *18*). Meanwhile, the leaves of *Petasites* species contain a low level of pyrrolizidine (*15*) and, moreover, have various antioxidants such as petasinophenol (*19*), flavonoid glycosides (*20*, *21*), phenylpropenoyl sulfonic acid (*22*), or fukinolic acid (*23*). In addition, very recently, the butanol fraction of *P. japonicus* leaves was found to contain a new furofuran lignan, which was named petaslignolide A (PA) (*24*).

The aim of present study is to examine the neuroprotective action of PA against oxidative damage in the brains of mice treated with kainic acid and, in addition, to determine whether PA is mainly responsible for the neuroprotective action of the butanol fraction from *P. japonicus* leaves extract.

MATERIALS AND METHODS

Materials. Kainic acid, glutathione reductase (type III from baker's yeast), reduced glutathione, oxidized glutathione (GSSG), 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). Pinoresinol glycoside was kindly supplied by Dr. Byung-Sun Min, Laboratory of Immunomodulator, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea.

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

Preparation of Vegetable Extracts. Washed and chopped leaves of fresh *P. japonicus* Maxim. were dried in a freeze-dryer (at −70 °C), and the dried leaves (1.4 kg) were extracted three times with methanol in a dark place (15 °C). Methanol extract (310 g), after concentration, was suspended in distilled water, and the suspension was partitioned with hexane, chloroform, ethyl acetate, and *n*-butanol, successively as reported previously (*24*). The *n*-butanol fraction (BMP) was dried and then redissolved in 10 volumes of *n*-butanol. After centrifugation (17000*g*, 10 min) of the mixture, the soluble fraction was dried under vacuum evaporation to give BMP-I.

Separation of Petaslignolide A from the Butanol Fraction. The active neuroprotective compound, which was isolated from the butanol fraction (BMP) using silica gel chromatography and C18 gel chromatography successively, was identified by LC-MS and NMR and named petaslignolide A (**Figure 1**), 2α-(4′-hydroxy-3′-methoxyphenyl)-6α-(4″-hydroxy-3″-methoxyphenyl)-8α-hydroxy-3,7-dioxabicyclo[3.3.0]-octane 4′-*O*-(β-D-glucopyranoside) (*24*).

Animal Experiments. Male ICR mice (20−23 g), 6−8 weeks of age, were housed in polycarbonate cages (five animals per cage) and fed unrestricted amounts of filtered water and pelleted commercial diet (Samyang Co.). 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 the *Guide for Care and Use of Laboratory Animals* of the National Institutes of Health (*25*). The mice were assigned randomly to treatment groups (eight mice/group) and were weighed individually every day. Mice were administered orally each sample (40−200 mg/kg, 5 mL/kg) suspended in saline or saline as vehicle using a gavage needle for 4 consecutive days before kainic acid injection; 30 min after the final administration, the animals were challenged by intraperitoneal (ip) injection (50 mg/kg, 3 mL/kg) of kainic acid, dissolved in 10 mM phosphate-buffered saline, pH 7.4 (*13*, *26*). Following the challenge with KA, the onset times of neurobehavioral activities such as tail arch, tremors or seizures, and the mortality of the animals were monitored for 60 min (*27*, *28*). Most of the animals exhibited tail arch and finally reached tremors, followed by seizures or death. Two days after the KA administration, the brain tissues of mice were removed after intracardial perfusion, under light anesthesia with ether, with cold saline to avoid blood contamination.

Measurement of Lipid Peroxidation in Brain Tissue. Brain tissue, rinsed with 0.15 M KCl solution containing 2 mM EDTA, was homogenized in 9 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 and then centrifuged (14000 rpm, 10 min), and then the absorbance of the supernatant was measured at 532 nm as previously described (*29*).

Determination of Total GSH in Brain Tissue. Brain tissue (0.27−0.30 g wet weight) was pulverized in a cooled ceramic percussion mortar with 6% metaphosphoric acid, and the mixture was centrifuged (27000*g*, 20 min) at 4 °C. Total GSH was determined enzymatically according to a published procedure (*30*) with a slight modification. To 0.02 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 of equilibration at 25 °C, the reaction was started by adding 1 unit of GSH reductase. The formation of 2-nitro-5-thiobenzoic acid was continuously recorded at 412 nm. The total amount of GSH in the samples was determined from a standard curve.

Assay of GSH Peroxidase and Reductase. Brain tissue was homogenized in 9 volumes of 20 mM phosphate buffer containing 0.1 M KCl, 1 mM EDTA, and 0.5% Triton X-100. The homogenate was centrifuged (15000*g*, 15 min), and the supernatant was recentrifuged (105000*g*, 30 min). The last supernatant was retained for enzymatic assays. The assay of GSH peroxidase activity was carried out as described previously (*31*). A mixture containing 0.1 M phosphate buffer (pH 7.0), 3 mM EDTA, 1 mM GSH, 0.1 mM NADPH, 2 units of GSH reductase, and 0.05 mL of supernatant was incubated for 3 min, and 0.01 mL of 10 mM cumene hydroperoxide was then added to the reaction mixture. GSH reductase activity was measured in a mixture containing 0.1 M phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM GSSG, 0.1 mM NADPH, and 0.05 mL of the supernatant (*31*).

Protein Determination. Protein was determined according to the method of Bradford (*32*) using bovine serum albumin as a standard.

Uptake of Evans Blue into the Brain. Evans blue (3 mL/kg, 2% in saline) was administered intravenously (iv) to mice, either PA-treated or vehicle-treated, 60 min before KA administration, and after 2 h, the brain tissue was taken and homogenized in 50 mM Tris buffer (pH 7.4); after centrifugation (100 000 rpm, 30 min) the absorbance of the supernatant of brain homogenate was measured at 615 nm (*33*).

Statistical Analyses. All statistical analyses were performed using an SAS program (*34*). 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 (SE). Statistical assessments were performed using ANOVA, followed by a post-hoc Duncan's multiple-range test for biochemical and neurobehavioral changes or

Table 1. Effect of Each Methanol Subfraction from Silica Gel Chromatography on Behavior and Mortality in Mice Injected with Kainic Acid[a]

group	tail arch (min)	tremors (min)	seizure (min)	death (min)	mortality (%)
kainic acid	7.2 ± 2.9a	12.7 ± 5.0a	16.1 ± 7.1a	22.0 ± 9.7a	75.0
+ 30%	13.4 ± 4.1b	17.1 ± 4.4b	37.1 ± 14.8b	44.5 ± 19.0b	25.0
+ 70%	11.5 ± 5.7a	17.0 ± 5.4ab	22.8 ± 10.8a	27.3 ± 14.6ab	50.0
+ 100%	9.5 ± 3.7a	13.0 ± 3.4a	16.0 ± 3.7a	22.3 ± 7.6a	87.5

[a] Mice ($n = 8$) were administered orally each subfraction (100 mg/kg) from silica gel column chromatography for 4 days before kainic acid injection, and then the behavioral signs were monitored for 60 min, onset time of behavioral signs, and incidence percentage. Any two means in the same row with different letters represent a significant difference at $P < 0.05$.

Table 2. Effect of Petaslignolide A or BMP-1 on Behavior and Mortality in Mice Injected with Kainic Acid[a]

group	tail arch (min)	tremors (min)	seizure (min)	death (min)	mortality (%)
kainic acid	6.6 ± 2.4a	9.5 ± 3.1a	12.5 ± 4.0a	16.5 ± 4.0	50.0
+ PA (40 mg/kg)	12.4 ± 3.4b	16.1 ± 4.1b	22.9 ± 5.7b	22.0	12.5
+ BMP-1 (200 mg/kg)	12.1 ± 3.5b	16.6 ± 4.8b	23.0 ± 7.0a	15.0	12.5

[a] Mice ($n = 8$) were administered orally PA or BMP-1 for 4 days before kainic acid injection, and the behavioral signs were monitored for 60 min. Values are means ± SE. Any two means in the same row with different letters represent a significant difference at $P < 0.05$. PA, petaslignolide A; BMP-1, subfraction of butanol extract.

Scheffe's test for histopathology (*35*). Statistical significance refers to results where $P < 0.05$ was obtained.

RESULTS

In the previous study (*14*, *24*) to find a fraction of the *P. japonicus* methanol extract expressing a potent antioxidant action, the ethyl acetate fraction was found to be more efficient than the butanol extract in scavenging the diphenylpicrylhydrazyl (DPPH) radical. However, despite its potent antioxidant action, the ethyl acetate fraction was observed to demonstrate no significant protection against kainic acid neurotoxicity in vivo experiment (*14*), in contrast to the butanol fraction, which showed a remarkable neuroprotection. Thus, it was supposed that the neuroprotective action of the butanol fraction of *P. japonicus* extract might not be related to the in vitro antioxidant potency. Accordingly, the butanol fraction was subjected to in vivo bioactivity-guided fractionation to isolate the neuroprotective compound capable of preventing KA neurotoxicity.

In Vivo Bioactivity-Guided Fractionation of the Neuroprotective Compound from Butanol Fraction. First, the neurotoxic effect of KA on the behavior of male mice was investigated. A single administration of kainic acid (50 mg/kg) caused a typical sustained seizure, which was evident 10 min after ip administration, with a tremor persisting for 20−30 min, in contrast to no seizure activity for the vehicle-treated group. When each subfraction (30, 70, and 100% methanol), obtained from silica gel column chromatography of the butanol fraction, was administered for 4 days before the exposure to KA (**Table 1**), the 30% methanol subfraction (100 mg/kg) was the most protective, with 25% mortality in contrast to 75% mortality in the group treated with KA alone. Moreover, the amelioration of behavioral signs was the most remarkable with 30% methanol subfraction.

Subsequently, the 30% subfraction from silica gel chromatography was further separated on a C_{18} column, and then each subfraction (I, II, III, IV, or V) was tested for the neuroprotective activity; subfraction II (100 mg/kg) was the most protective (mortality, 12.5%), in contrast to 87.5% mortality in the group treated with KA alone. Finally, the neuroprotective compound (petaslignolide A) was isolated as a major component from subfraction II of C_{18} column chromatography (*24*).

Neuroprotective Action of Petaslignolide A. When the neuroprotective activity of PA, dissolved in dimethyl sulfoxide (DMSO), was examined for the neuroprotective action, it was observed that PA (40 mg/kg) was effective in reducing the mortality (**Table 2**); the mortality in PA/KA-treated mice group decreased to 12.5%, in contrast to 50% in the vehicle/KA-treated group. In addition, PA remarkably ameliorated the behavioral signs caused by KA. In a comparative study, BMP-I, a subfraction of the butanol fraction, also expressed a similar neuroprotection, but required a higher dose (200 mg/kg). Thus, it is suggested that PA may be a principal neuroprotective compound in the butanol fraction of *P. japonicus* leaves. In a separate experiment, where the dose of PA was varied, the increase of the dose of PA to 60 mg/kg did not further enhance the protective action and the decrease of the dose to 20 mg/kg was less protective. Separately, although pinoresinol glycoside, another lignan compound, was tested for neuroprotective action, it showed no significant neuroprotective action (unpublished data).

To see whether the neuroprotective action of PA was related to the prevention against oxidative stress in the brain tissue of mice intoxicated with KA, we examined the effect of PA on the level of thiobarbituric acid-reactive substances (TBARS), a biochemical marker of lipid peroxidation, in the brains of mice administered KA (50 mg/kg). **Figure 2** shows that the TBARS value was increased to 168% of control value ($P < 0.05$) in the homogenate of the whole brain of mice treated with KA. Meanwhile, PA (40 mg/kg) reduced the TBARS value, which was enhanced by KA challenge, to the level of control group ($P < 0.05$). Also, a similar result was observed with BMP-I (200 mg/kg). These results suggest that at least some part of the neuroprotective action of PA may be due to its preventive action against oxidative stress in the brain.

In a related experiment, we examined the effect of PA on the level of total glutathione, another biomarker of oxidative damage, in the brains of mice administered KA (50 mg/kg). As shown in **Figure 3**, the administration of PA (40 mg/kg) elevated the level of total GSH in the brain cytosol from 73% in the KA-treated group to 97% of control level, further confirming the notion that the neuroprotective effect of PA may be related to its prevention against oxidative stress in the brain.

In a further endeavor to see the mechanism responsible for the inhibition of oxidative stress by PA (40 mg/kg), the change in glutathione-related enzymes such as GSH peroxidase and GSH reductase in the PA/KA-treated group was examined. **Figure 4A** indicates that the treatment with PA successfully restored the loss of GSH peroxidase activity, which was reduced

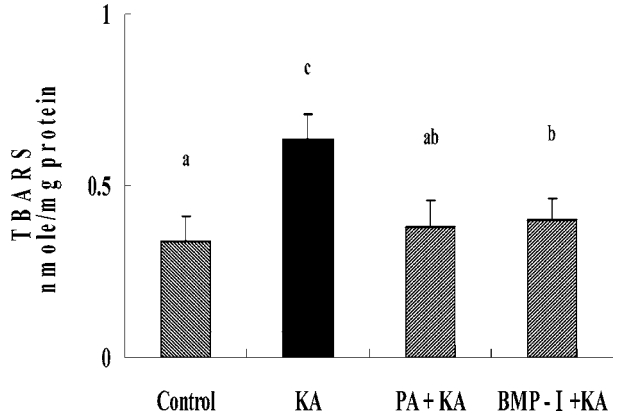

Figure 2. Effect of petaslignolide A or BMP-I on TBARS in brain tissue of mice administered kainic acid. Male ICR mice received by gavage petaslignolide A (40 mg/kg) or BMP-1 (200 mg/kg) consecutively for 4 days, and then kainic acid was administered 1 day before sacrifice. Brain tissue was homogenized in PBS buffer, and the whole homogenate was used for the determination of TBARS value. Values with the same letter are not significantly different ($P < 0.05$). Values are means ± SE of three determinations from four to eight mice. KA, kainic acid; PA, petaslignolide A; BMP-1, subfraction of butanol extract.

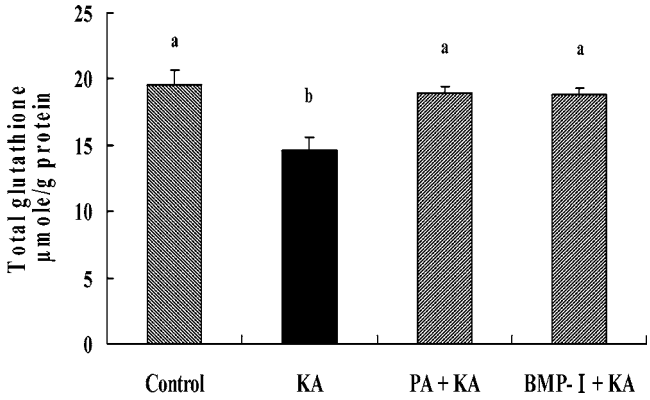

Figure 3. Effect of petaslignolide A or BMP-I on total glutathione level in the brain tissue of mice administered kainic acid. Brain tissues were pulverized in a cooled ceramic percussion mortar with 6% metaphosphoric acid, and the supernatant, after centrifugation, was used for the determination of GSH. Values with the same letter are not significantly different ($P < 0.05$). Values are means ± SE of three determinations from four to eight mice. KA, kainic acid; PA, petaslignolide A; BMP-1, subfraction of butanol extract.

by KA treatment, to control level. In contrast, there was no significant difference of GSH-reductase activity among the mice of the vehicle-treated group, KA-treated group, and PA/KA-treated group (**Figure 4B**), suggesting that under the experimental conditions used, the neurotoxicity of KA may be insufficient to significantly alter the level of GSH-reductase activity.

In a separate study, the direct effect of PA on KA neurotoxicity was examined. For this purpose, the compound (40 mg/kg) was only once administered to mice 45 min before KA injection, and its protective effect was determined. However, the neuroprotective action was not observed with the singular administration of PA, excluding the notion that PA itself may directly decrease the neurotoxicity of KA.

Effect of Petaslignolide A on the Induction of GSH or GSH-Related Antioxidant Enzymes. To see whether the neuroprotective action of PA may be related to the induction

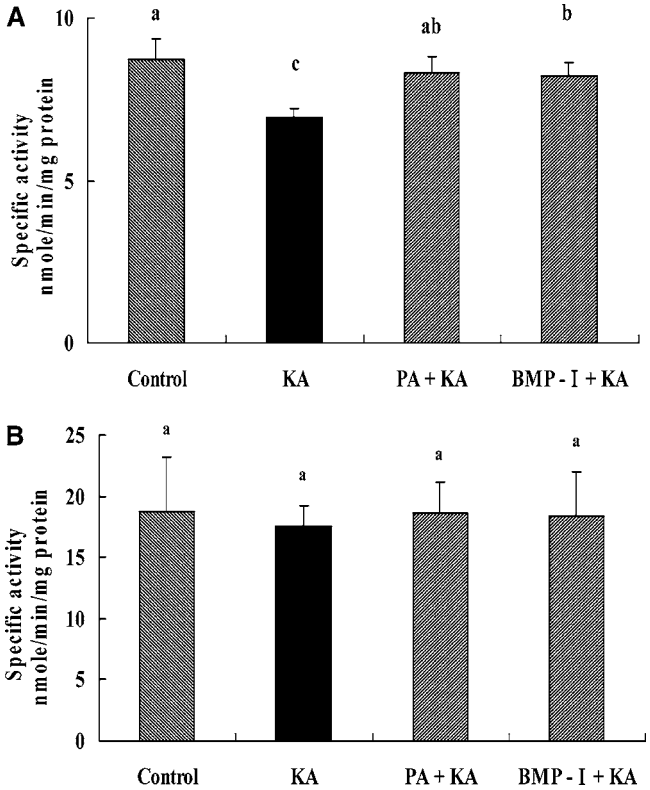

Figure 4. Change of glutathione peroxidase activity (**A**) or glutathione reductase activity (**B**) in the brain tissue of mice administered a combination of kainic acid and petaslignolide A or BMP-I. Values are mean ± SE of three determinations from four to eight mice. Values with the same letter are not significantly different ($P < 0.05$).

of GSH or GSH-related antioxidant enzymes, PA (40 mg/kg) was orally administered to mice for 4 days, and then the changes in GSH, GSH peroxidase, or GSH reductase in the brain tissue of mice were determined. However, there was no significant alteration of GSH content as well as glutathione-related enzymes, GSH peroxidase and GSH reductase, excluding the possibility that the neuroprotective effect of PA may be related to the induction of GSH and glutathione-related enzymes in brain tissue (unpublished data).

Effect of Petaslignolide A on Oxidative Damage of the Blood−Brain Barrier Due to Kainic Acid-Induced Seizures. Separately, the protective effect of PA on damage to the blood−brain barrier caused by KA was examined. For this purpose, Evans blue (3 mL/kg, 2% in saline) was iv administered to mice, either the PA-treated group or the vehicle-treated group, 30 min before KA administration, and the brain tissue was analyzed for Evans blue retaining. When the tissue was homogenized, and the absorbance of the supernatant of the brain homogenate was measured at 615 nm (**Figure 5**), the amount of Evans blue retained in the brain following KA intoxication was significantly higher as compared to control. Meanwhile, pretreatment with PA significantly reduced the Evans blue level in the KA-treated group compared to the control level. Thus, PA is assumed to prevent against oxidative damage of the blood−brain barrier in KA-treated mice.

Effect of Petaslignolide A on Body and Brain Weights. In the following experiment, PA was administered by gavage to mice before exposure to KA. The administration with PA (40 mg/kg) caused no significant change in body or brain weight (**Table 3**).

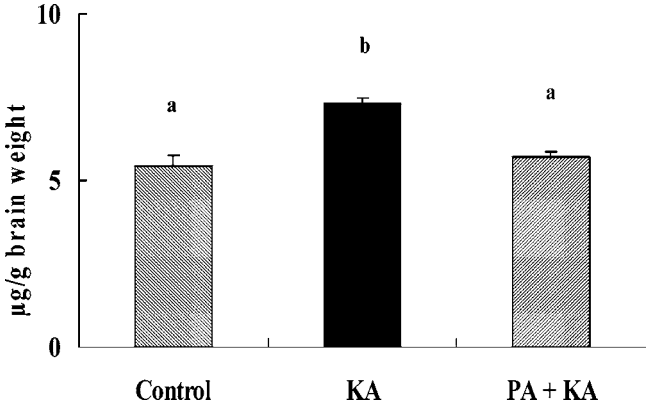

Figure 5. Effect of petaslignolide A on Evans blue uptake into the brains of mice administerd kainic acid. The whole brain was homogenized with 50% TCA, and the absorbance of supernatant after centrifugation was measured at 615 nm. Values are mean ± SE of three determinations. Values with the same letter are not significantly different ($P < 0.05$).

DISCUSSION

Although the brain has endogenous antioxidants such as GSH and melatonin, the ability of these antioxidants to combat oxidative stress in the brain tissue is limited in KA-induced neurotoxicity, which is characterized by the remarkable change in the levels of GSH and TBARS in brain tissue. Meanwhile, the administration of GSH or melatonin had been reported to protect against KA-induced neuropathological changes in rat brain (*4*, *10*), suggesting the value of a pharmacological strategy directed toward the maintenance of reducing capacity in brain tissue. For this purpose, the use of phenolic antioxidants could be a potential approach. However, despite lots of reports on the antioxidant action of phenolic compounds, there have been a limited number of reports on the neuroprotective action of phenolic compounds in vivo (*10*, *36*); even *trans*-resveratrol, which successfully prevented lipid peroxidation in brain tissue, failed to maintain the GSH level. This may suggest that the neuroprotective action of phenolic compounds may be limited by factors other than antioxidant potency. Recently (*36*−*39*), there is an emerging line of evidence on the antioxidant action of lignan compounds, another type of phenolic compounds. Some of them expressed a good protection against the cytotoxicity caused by excitotoxins in vitro (*38*). In the present study, PA, a new lignan anitioxidant, expressed a remarkable neuroprotective action against KA neurotoxicity. Furthermore, it is supposed that the lignan compound may be mainly responsible for the neuroprotective action of the butanol fraction of *P. japonicus*. This is well supported by an additional finding that the subfraction containing PA is much more neuroprotective than the other subfractions from the butanol fraction of *P. japonicus* extract.

Concerning the mechanism for the neuroprotective action of PA, it is possible to think that such a protective action of PA may be related to its prevention against oxidative stress as demonstrated from its GSH-sparing activity as well as a preventive action against lipid peroxidation in brain tissue. In addition, the level of GSH peroxidase activity was restored to control level after PA treatment. Moreover, PA reduced the uptake of Evans blue into brain tissue of KA-treated mice, in agreement with its protective effect on oxidative damage of the blood−brain barrier. Nevertheless, our additional finding does not support the inductive effect of PA on antioxidant systems in brain tissue.

It is noteworthy that PA expressed an apparent neuroprotective action despite its low antioxidant action in the in vitro system. This might be explained by the assumption that PA is metabolized to an active compound, which may exert an antioxidant action in the brain. In support of this, the singular administration of PA 30 min before KA challenge failed to show any neuroprotective action. A recent review indicates that furfuran or butyrofuran lignan compounds express antioxidant action after the metabolic conversion to enterolignans (*39*, *40*). Similarly, it is likely that PA is metabolized to a type of enterolignan, which may be further subjected to deglycosylation by a β-glucosidase. It should be noted that the butanol fraction of *P. japonicus* Maxim. is the most neuroprotective among edible plant extracts tested in our study (*14*). Generally, the antioxidant compounds are known to widely distribute in the butanol fraction and/or ethyl acetate fraction of plant extracts. Previous studies indicated that the ethyl acetate fraction from *Petasites* species extracts contained various bioactive sesquiterpene derivatives, such as petasin, isopetasin, or *S*-petasin, which possessed various bioactivities (*15*−*22*). In addition, petasiphenol, a polyphenol in the ethyl acetate fraction, was found to show radical-scavenging activity (*19*). Despite its various bioactivities, the ethyl acetate fraction of *P. japonicus* was not comparable to PA in preventing KA neurotoxicity. Even, *S*-petasin, a Ca^{2+}-blocking sesquiterpene, failed to show a remarkable protective action against KA neurotoxicity (*17*). Thus, the constituents present in the ethyl acetate fraction of *Petasites* species may be far from the neuroprotective action.

Some polyphenols from plant extracts are known to show a neuroprotective effect in vitro; some flavonoids such as patuletin, isolated from the butanol fraction of *Inula britannica*, prevented a decrease in the amount of GSH as well as activities of antioxidant enzymes in glutamate-injured cortical cells (*41*). Arctigenin, a butyrolactone lignan, was found to prevent glutamate-induced toxicity in primary cultured rat cortical cells (*38*). Additionally, caffeinylquinic acid, an antioxidant phenol from rough aster extract, exhibited a neuroprotective effect in the PC12 cells exposed to KA (*12*). Nevertheless, there has been no report concerning the neuroprotective action of these phenolic antioxidants in in vivo systems. Furthermore, although the extract of *Petasites* species had been reported to contain various polyphenols such as caffeic acid, fukinolic acid, petasiformin A, kaempferol 3-*O*-(6′-acetyl)-β-glucopyranoside, or quercetin 3-*O*-(6′-acetyl)-β-glucopyranoside (*19*−*22*), the subfractions containing these phenolic compounds did not express a remarkable neuroprotective action. In fact, our unpublished data show that quercetin or gallocatechin gallate had no significant

Table 3. Change of Body Weight and Brain Weight of Mice Administered Kainic Acid and Peaslignolide A[a]

treatment	initial body weight (g)	final body weight (g)	body weight (g)	brain weight (g)
control	20.7 ± 0.5 NS	24.3 ± 0.5 NS	3.5 ± 0.4 NS	0.280 ± 0.02 NS
KA (50 mg/kg)	21.3 ± 0.8	24.4 ± 0.3	3.1 ± 1.0	0.280 ± 0.01
PA (40 mg/kg)	21.0 ± 0.6	24.4 ± 0.4	3.7 ± 0.7	0.294 ± 0.02
KA + PA	21.2 ± 0.7	24.3 ± 0.6	3.3 ± 0.7	0.281 ± 0.01

[a] Values are means ± SE of determinations from four to eight mice. NS, not significant at $p < 0.05$; KA, kainic acid; PA, petaslignolide A.

neuroprotective action against KA neurotoxicity in vivo. Even pinoresinol glycoside (*37*), belonging to a furfuran lignan, did not exhibit a significant neuroprotective action. All of this suggests that the neruoprotective action could not be explained by the structure of antioxidant compounds only. More important, the neuroprotective compounds must pass through the blood−brain membrane to be effective. Although the active species of PA for the neuroprotective action was not identified here, it is supposed that the active species would be an antioxidant metabolite of PA. Alternatively, it is possible that the neuroprotective action of the PA metabolite might be due to other activities such as the restoration of the imbalance of glutamate or GABA content (*1*−*3*) or the ability to antagonize the binding of KA to glutamate receptors (*42*).

A key finding of the present study is that PA in the butanol fraction of the leaves of *P. japonicus*, an edible plant in Korea and Japan, shows a remarkable neuroprotective action against KA excitoxicity. Thus, the butanol fraction of *Petasites* species extract, containing PA, might be useful in preventing neurodegenerative disorders caused by oxidative stress. Such a bioactivity of PA, present in the butanol extract, may add to the usefulness of *Petasites* species extracts as functional foods in addition to the use of the ethyl acetate fraction (Ze 339) of *P. hybridus* as an antimigrane or antiasthma agent (*15*, *16*).

ABBREVIATIONS USED

BMP-1, butanol subfraction of methanol extract from *Petasites japonicus*; GSH, reduced glutathione; KA, kainic acid; TBARS, thiobarbituric acid-reactive substances; DTNB, 5,5′-dithionitrobenzoate; PA, petaslignolide A.

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