



Effects of Single and Combined Administration of Fermented Barley Extract and γ -Aminobutyric Acid on the Development of Atopic Dermatitis in NC/Nga Mice

Hideki HOKAZONO,^{1,2,†} Toshiro OMORI,¹ and Kazuhisa ONO²

¹Research Laboratory, Sanwa Shurui Co., Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan

²Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8530, Japan

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We examined the effects single and combined administration of fermented barley extract P (FBEP), prepared from barley-*shochu* distillery by-products, and γ -aminobutyric acid (GABA) on the development of atopic dermatitis (AD)-like skin lesions in NC/Nga mice. Single administration of FBEP and GABA dose-dependently reduced the development of AD-like skin lesions in mice. GABA reduced the development of AD-like skin lesions by suppressing serum immunoglobulin E (IgE) and splenocyte interleukin (IL)-4 production, while FBEP reduced skin lesions without affecting the IgE or cytokine production. However, in mice with induced AD-like skin lesions, combined administration of FBEP and GABA decreased serum IgE levels and splenic cell IL-4 production, and increased splenic cell interferon- γ production. These results suggest that combined administration of FBEP and GABA alleviated AD-like skin lesions in the NC/Nga mice by adjusting the Th1/Th2 balance to a Th1-predominant immune response.

Key words: fermented barley extract; γ -aminobutyric acid; atopic dermatitis; interleukin-4; interferon- γ

Atopic dermatitis (AD) is an illness characterized by pruriginous eczema as its main morbid process, in which aggravation and abatement are repeated, and most AD patients present an atopic disposition.¹⁾ Allergic reactions to antigens, namely immunologic disorders and disorders of skin barrier function such as atopic dry skin, are important factors in the development of AD. Therefore, based on dualistic thought, the control of inflammation, a reduction in pruritus, and skin care are important in the treatment of AD. Moreover, since environment factors and mental stress are complicating issues in the development and aggravation of dermatitis, it is important to pay attention to such conditions. While foods contain allergens, they also contain ingredients that provide preventive and palliative effects on allergic reactions. In recent years, anti-allergic foods utilizing the functionality of such food ingredients have been attracting attention, and lactic acid bacteria and plant polyphenols have been the main focus of reports.²⁻⁵⁾

We have studied the effective application of *shochu* distillery by-products (SDB) and have reported the effects of fermented barley extract (FBE), obtained from a barley-SDB, in preventing fatty liver induction by orotic acid and D-galactosamine-induced liver injury.^{6,7)} Additionally, oral administration of FBEP (the adsorbed fraction of FBE on the hydrophobic resin) in mice resulted in reduced oxidative stress by controlling glutathione-redox.⁸⁾ On the other hand, Iguchi *et al.*^{9,10)} reported that administration of FBE to mice with ovalbumin-sensitized rhinitis reduced the increase in frequency of sneezing and nose-scratching. Furthermore, the development of AD-like skin lesions in NC/Nga mice was reduced by alterations in the cytokines involved in chronic inflammation, such as interferon (IFN)- γ and IL-17. In addition, epidemiological studies have indicated that a low incidence of asthma was observed in a population with a high intake of flavonoids.¹¹⁾ Hence it is expected that the antioxidative and anti-inflammatory actions of FBEP (polyphenol fraction of FBE) will result in allergy-reducing effects.

On the other hand, several expression aberrations of neurotransmitters have been reported in the skin of AD patients.¹²⁻¹⁴⁾ It was reported that noradrenalin (NA), a major neurotransmitter involved in the control of the vasoconstrictive motion of human skin, was most enriched in the skin of AD patients and might be one of the pathological, aggravating factors of AD by increasing inflammatory cytokine production.¹⁵⁾ Kimura *et al.*¹⁶⁾ and Hayakawa *et al.*¹⁷⁾ confirmed that GABA showed a hypotensive effect in spontaneously hypertensive rats by suppressing the release of NA in the peripheral sympathetic nerves. In addition, it was found that GAD67, a GABA-producing enzyme, was present in the dermal fibroblasts, and that GABA was involved in the activation of cellular function, for example in promoting the production of hyaluronic acid and the antioxidant glutathione.¹⁸⁾ Thus GABA might exhibit anti-inflammatory and AD-mitigating effects by improving skin barrier function.

The present study examined the influence of single and combined administration of FBEP and GABA on

[†] To whom correspondence should be addressed. Tel: +81-978-33-3844; Fax: +81-978-33-5811; E-mail: hokazono-h@kokuzo.co.jp

Abbreviations: SDB, *shochu* distillery by-products; FBE, fermented barley extract; GABA, γ -aminobutyric acid; AD, atopic dermatitis; IgE, immunoglobulin E; IL, interleukin; IFN- γ , interferon- γ ; NA, noradrenalin; PiCl, picryl chloride; PSL, prednisolone; s-IgA, secretory IgA

the progress of AD-like skin lesions in NC/Nga mice by continuous application of the hapten picryl chloride (PiCl).

Materials and Methods

Materials. Fresh barley-SDB (Sanwa Shurui, Oita, Japan), separated during vacuum distillation, was prepared using pearled barley (barley pearled to approximately 65% of its initial weight) as the raw material. Preparation of FBE and FBEP were as follows: Barley-SDB was filtered with a stainless-steel mesh net (1 mm). The extracted liquid was then filtered using a ceramic filter (porosity 0.2 µm) to remove solid residues, such as plant cell walls and microbial cells. Subsequently, the aqueous solution (FBE) was freeze-dried and mixed with 50% w/w water-soluble dextrin (Pinedex 100™; Matsutani Chemical Industry, Hyogo, Japan) as an excipient. A 10-liter volume of FBE, before concentration and drying, was subjected to an Amberlite FPLC66™ (Rohm and Haas, Philadelphia, USA) column (80 × 10 cm I.D.) equilibrated with water. After washing with water, the trapped material was eluted with 1 wt-% NaOH. The eluted fraction was subjected to an Amberlite IRC76™ hydrogen form (Rohm and Haas) column. The pass-through fraction was freeze-dried. As a result, approximately 100 g of freeze-dried sample (FBEP) was obtained from 10 kg of pearled barley. The composition of the FBEP was as follows: 41.8% carbohydrate, 39.2% protein, 8.8% dietary fiber, 0.3% fat, 3.7% ash, 6.2% water, and 8.5% polyphenol.

GABA (>90% purity) was produced by natural fermentation using the lactic acid bacterium *Enterococcus* sp. FC 301 (Lacto-Fermented Barley GABA, Barley Fermentation Technologies, Oita, Japan).

Animals. Female NC/Nga mice (7 weeks old) were obtained from Charles River Japan (Atsugi, Japan). The mice were housed in polycarbonate cages. The animal room was kept at $23 \pm 2^\circ\text{C}$ and the relative humidity at 30–80%. Room lighting consisted of a 12-h light/dark cycle. The mice were given free access to diet (CRF-1, Oriental Yeast, Tokyo, Japan) and water. They were subjected to experimentation after a 1-week period of quarantine and domestication. The animal experiments were performed in accordance with the “Standards Relating to the Care and Management of Experimental Animals (Notification No. 6, March 27, 1980, of the Prime Minister’s Office, Japan, revised May 28, 2002)” and “The Japan Act on the Welfare and Management of Animals (up to the revisions of Act No. 50 of 2006).” In order to make the average body weight and standard deviations almost equal among the groups, the mice were divided into 10 groups: a normal control group (untreated), a control group (PiCl-treated), two FBE groups (the diets contained FBE at 2% or 10%), two FBEP groups (the diets contained FBEP at 0.5% or 2.5%), two GABA groups (the diets contained GABA at 0.1% or 0.25%), an FBEP-GABA group (the diet contained 2.5% FBEP and 0.25% GABA), and a prednisolone (PSL) group (3 mg/kg/d was administered orally using a stomach sonde) ($n = 8$ each group). The CRF-1 diet was given to the normal control, control, and PSL groups. The test sample groups mixed each test sample to achieve the aforesaid additive rate in the CRF-1 diet. Food consumption (g/d) was observed and body weights were measured once a week. The normal control group received no treatment, and the other seven groups were treated with PiCl, as described below.

AD model mice. The abdominal and dorsal skin of the NC/Nga mice was shaved, and the shaved skin and sole of the foot were sensitized with 150 µl of 5% PiCl in an ethanol and acetone mixture (4:1). On day 4 after sensitization, the left auricle was challenged with 30 µl of 0.8% PiCl dissolved in olive oil. Challenge with 0.8% PiCl was repeated once a week for 10 weeks. Auricular thickness was measured every week with a digital thickness gauge (Peacock G-7C type, Ozaki MFG, Tokyo).

Measurement of serum IgE. Blood was collected by exsanguination from the heart at necropsy. Serum samples were obtained by centrifugation and stored at -80°C until use. The total serum IgE level was determined by ELISA (Shibayagi, Gunma, Japan).

Measurement of cytokine production. All the mice were sacrificed and the splenocytes were collected. The splenocytes were washed with RPMI-1640 medium with 10% fetal bovine serum, and then suspended at a concentration of 1.2×10^7 cells/ml.

The cells were cultured in a 24-well culture plate with anti-CD3 mAb (2 µg/ml) at 37°C for 48 h in a 5% CO_2 incubator. The culture supernatant was collected by centrifugation and stored at -80°C until assay. The concentrations of IFN- γ and IL-4 were determined with an ELISA kit (eBioscience, San Diego, CA).

Statistical analysis. Data were expressed as means $\pm$ SE. Student’s *t*-test was used to detect significant differences between the normal control group and the control group. One-way ANOVA with Bonferroni/Dunn’s *post hoc* test was used to test for significant differences between groups. The *p* values less than 0.05 were considered statistically significant.

Results

No significant differences between the average food intakes of the groups were observed during the study period. Table 1 shows the changes in body weight of the groups during the study period. The body weight of the PSL group was significantly lower than the PiCl-treated control group 5 weeks after the start of sensitization until the end of the study period. For the other groups, no significant differences were observed among the groups.

Table 2 shows the changes in auricular thickness for the groups during the study period. No change in the thickness of the auricle of the normal control group was observed, whereas the auricle of the PiCl-treated control group showed a significant increase in thickness as compared with the normal control group 1 week after the onset of sensitization. Although the auricular thickness of each group administered the test substances increased at each time of repeated induction in the same manner as in the PiCl-treated control group, the increase in thickness was reduced as compared to the PiCl-treated control group 1–3 weeks after the onset of sensitization to the end of the study period. The dose-dependent, thickness-reducing effect of each test substance (FBE, FBEP, and GABA) was confirmed, but no additive/synergistic effects of combined administration of FBEP and GABA were observed. The thickness of the auricular area of the PSL group was further reduced as compared to each group administered the test substances, and was significantly thinner than the PiCl-treated control group throughout the study period.

Figure 1 shows the serum IgE levels of the various groups. The PiCl-treated control group showed significantly higher serum IgE levels than the normal control group under sensitization and 10 weeks of induction treatment. Compared to the PiCl-treated control group, the serum IgE of the PSL group showed significantly lower levels. The other groups administered either of the test substances showed no significant differences, but the serum IgE levels of the GABA (high dose) and FBEP-GABA groups tended to be low.

Figure 2A shows splenocyte IL-4 production by the various groups. The PiCl-treated control group showed significantly higher levels of splenocyte IL-4 than the normal control group, whereas the PSL group showed significantly lower levels than the PiCl-treated control group. The GABA-administered groups had a dose-dependent tendency to show lower splenocyte IL-4

Table 1. Changes in Body Weight of NC/Nga Mice in the Various Groups after Initial Sensitization

Group	(g)	Weeks after sensitization										
		0	1	2	3	4	5	6	7	8	9	10
Normal control	Mean	18.1	19.4	20.2	20.6	21.3	22.0	22.3	22.7	23.1	23.3	23.7
	SE	0.4	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.3	0.4
Control	Mean	18.1	19.4	19.5	19.7	20.5	20.7	21.1	21.4	21.6	21.9	22.5
	SE	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.4
FBE (2%)	Mean	18.1	19.9	20.8	21.2	22.1	22.5	22.5	23.1	23.2	23.9	24.1
	SE	0.4	0.5	0.4	0.4	0.5	0.6	0.5	0.5	0.5	0.5	0.5
FBE (10%)	Mean	18.1	18.3	20.0	20.6	21.5	21.7	21.7	22.0	22.1	22.5	23.0
	SE	0.6	0.8	0.5	0.7	0.6	0.5	0.5	0.5	0.5	0.5	0.6
FBEP (0.5%)	Mean	18.7	18.9	20.1	20.6	21.4	21.7	22.1	22.2	22.7	23.0	23.3
	SE	0.5	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.5	0.5	0.7
FBEP (2.5%)	Mean	18.8	19.3	20.6	21.2	21.8	21.8	22.4	22.5	22.6	22.9	23.4
	SE	0.4	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7
GABA (0.1%)	Mean	18.3	18.9	19.9	20.5	21.2	21.5	21.8	22.0	22.4	22.7	23.2
	SE	0.3	0.7	0.4	0.5	0.4	0.5	0.5	0.5	0.5	0.6	0.6
GABA (0.25%)	Mean	18.5	18.5	19.6	20.2	20.8	21.3	21.8	22.0	22.4	22.6	22.7
	SE	0.5	0.8	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4
FBEP-GABA	Mean	18.3	18.8	20.2	20.6	21.2	21.4	21.7	21.9	22.1	22.4	22.8
	SE	0.3	0.6	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.2
PSL	Mean	18.4	18.5	19.1	19.7	20.0	19.9	20.3	20.4	20.3	20.8	21.0
	SE	0.3	0.5	0.4	0.4	0.5	0.4*	0.4*	0.4**	0.4**	0.5**	0.5**

Body weight was measured once a week.

* $p < 0.05$ and ** $p < 0.01$, significant difference as compared to the control group.

Table 2. Effects of Consecutive Administration of Test Substances on Ear Thickness Induced by PiCl Application in NC/Nga Mice (n = 8/group)

Group	($\times 10^{-3}$ mm)	Weeks after sensitization										
		0	1	2	3	4	5	6	7	8	9	10
Normal control	Mean	165.4	165.1	164.9	163.7	163.3	162.0	161.6	163.0	163.9	164.3	165.3
	SE	1.0	1.0	1.6	0.8	0.7	0.6	0.5	0.5	0.3	0.4	0.3
Control	Mean	164.8	169.6	177.4	194.4	203.9	217.8	233.1	249.9	268.5	281.9	291.9
	SE	2.1	1.6 [#]	2.6 ^{##}	2.4 ^{###}	2.0 ^{###}	4.8 ^{###}	6.3 ^{###}	8.9 ^{###}	10.5 ^{###}	11.0 ^{###}	12.9 ^{###}
FBE (2%)	Mean	169.8	167.5	172.5	182.1	191.8	206.1	212.1	221.1	238.4	246.9	257.0
	SE	1.4	0.8	2.8	1.9 ^{***}	2.6 ^{***}	4.2 [*]	6.0 [*]	5.2 [*]	6.4 [*]	5.8 [*]	6.9 [*]
FBE (10%)	Mean	166.4	166.4	174.7	184.1	190.6	199.4	206.9	212.0	227.0	240.7	249.1
	SE	1.0	0.6	2.6 [*]	1.4 ^{**}	1.0 ^{***}	2.3 ^{**}	4.8 ^{**}	6.1 ^{***}	7.8 ^{**}	8.2 ^{**}	9.1 ^{**}
FBEP (0.5%)	Mean	163.0	164.1	172.1	177.1	183.9	194.0	204.3	209.1	230.4	242.5	249.4
	SE	0.9	0.8	2.1	1.1 ^{***}	1.4 ^{***}	3.4 ^{***}	6.0 ^{***}	4.8 ^{***}	5.7 ^{**}	6.2 ^{**}	6.1 ^{**}
FBEP (2.5%)	Mean	163.1	164.0	173.3	177.9	185.4	192.6	197.1	205.0	217.0	231.3	234.0
	SE	0.8	1.0	3.3	1.8 ^{***}	2.4 ^{***}	4.3 ^{***}	6.4 ^{***}	8.7 ^{***}	10.0 ^{***}	9.6 ^{***}	12.3 ^{***}
GABA (0.1%)	Mean	163.9	164.9	166.6	175.3	180.6	184.8	190.8	207.0	226.3	237.8	245.6
	SE	1.1	0.6 ^{**}	1.8 ^{**}	1.9 ^{***}	1.8 ^{***}	2.4 ^{***}	3.6 ^{***}	5.5 ^{***}	5.6 ^{***}	6.1 ^{**}	5.3 ^{**}
GABA (0.25%)	Mean	164.8	164.3	165.9	175.3	179.4	185.6	191.1	203.4	221.0	231.3	238.3
	SE	0.8	0.9 ^{**}	0.7 ^{**}	1.8 ^{***}	1.6 ^{***}	3.1 ^{***}	4.1 ^{***}	7.0 ^{***}	7.2 ^{***}	10.8 ^{***}	11.1 ^{***}
FBEP-GABA	Mean	164.6	165.3	166.9	174.8	183.6	189.0	191.8	203.4	220.5	232.5	243.6
	SE	1.0	0.9 [*]	1.5 ^{**}	1.5 ^{***}	1.0 ^{***}	2.3 ^{***}	3.8 ^{***}	5.3 ^{***}	6.6 ^{***}	7.1 ^{***}	5.7 ^{**}
PSL	Mean	166.8	164.9	162.5	165.1	170.6	169.0	167.6	171.3	179.8	191.8	195.4
	SE	0.6	0.9 ^{**}	2.0 ^{***}	2.1 ^{***}	2.2 ^{***}	1.9 ^{***}	2.0 ^{***}	3.1 ^{***}	4.3 ^{***}	7.5 ^{***}	8.1 ^{***}

Auricular thickness was measured once a week.

[#] $p < 0.05$, ^{##} $p < 0.01$, and ^{###} $p < 0.001$, significant difference as compared to the normal control group.

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$, significant difference as compared to the control group.

levels, while the high-dose groups showed a significant reduction in IL-4 levels as compared to the PiCl-treated control group. The FBEP-GABA group showed significantly lower IL-4 levels than the PiCl-treated control group.

Figure 2B shows the splenocyte IFN- γ production of each group. The PiCl-treated control group showed significantly lower IFN- γ levels than the normal control group. The single administration group for each test substance (FBE, FBEP, and GABA) showed almost no

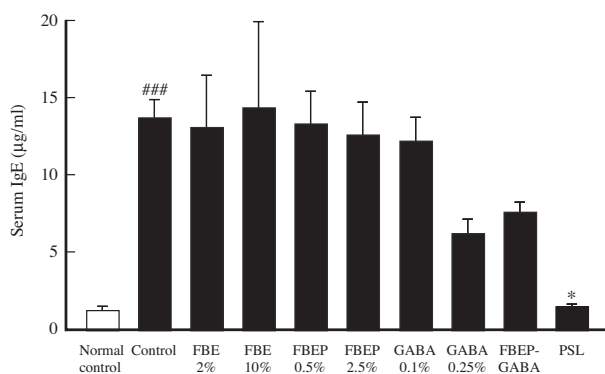


Fig. 1. Effects of Test Substances on Serum IgE Levels in NC/Nga Mice.

Sera were collected at 10 weeks after the initial challenge with PiCl. Total IgE levels were determined by ELISA. Each bar represents the mean $\pm$ SE for eight mice. ### $p < 0.001$, significant difference as compared to the normal control group. * $p < 0.05$, significant difference as compared to the control group.

difference in INF- γ level compared to the PiCl-treated control group, but the INF- γ levels in the FBEP-GABA group was significantly higher than in the PiCl-treated control group, but its level was found to be equal to those of the normal control and PSL groups.

Discussion

The morbid state of AD is thought to be the result of allergic reactions of IgE-mediated type I (immediate and delayed type) and type IV (delayed type),¹⁹ and an animal model in which individual type or mixed type allergic reactions develop can be used to study AD. The NC/Nga mice used in the present study spontaneously develop dermal symptoms similar to human AD,²⁰ and it has been reported that repeated application of hapten to mice under a specific pathogen-free environment can result in the development of the same dermatitis as in spontaneous development.²¹ In the present study, PiCl was used as the hapten. It was applied to the auricles of NC/Nga mice once a week for 10 weeks continuously. The development of dermatitis was accompanied by an increase in serum IgE levels and by increased IL-4 and decreased INF- γ levels in the splenocytes. According to a report by Gao *et al.*,²² it was confirmed that continuous application of mite antigens to the auricles of NC/Nga mice once a week for 4 weeks continuously resulted in increased serum IgE levels and in increased IL-4 and decreased INF- γ mRNA expression levels in the auricular tissue. We believe that their report corresponds well to the results of the present study.

Although single administration of each of the test substances (FBE, FBEP, GABA) to AD-like skin lesions resulted in thickened auricles during the study period, the auricles were significantly and strongly reduced in thickness as compared to the PiCl-treated control group, with strength of reduction in the order of FBEP > GABA > FBE. Since GABA was observed to reduce the increase in serum IgE and splenocyte IL-4 production, it was thought that adjustment of the Th1/Th2 balance to a Th1-predominant position reduced the AD-like skin lesions. However, since INF- γ was unaffected by single administration of GABA, the skin

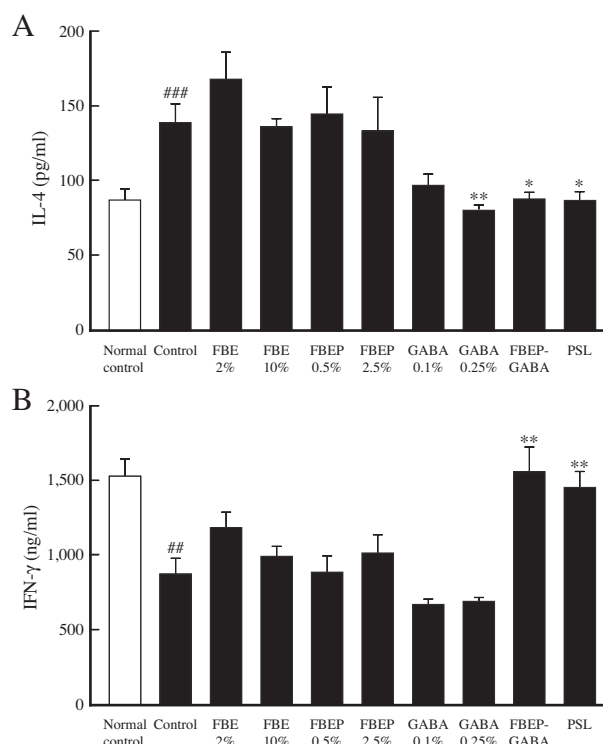


Fig. 2. Effects of Test Substances on Cytokine, IL-4 (A), and INF- γ (B), Production by Spleen Cells in NC/Nga Mice.

Splenocytes were prepared from NC/Nga mice 10 weeks after the initial challenge with PiCl. Cells (1.2×10^7 cells/ml) were cultured in a 24-well plate with anti-CD3 mAb ($2 \mu\text{g/ml}$) at 37°C for 48 h. Each bar represents the mean $\pm$ SE for eight mice. ## $p < 0.01$ and ### $p < 0.001$, significant difference as compared to the normal control group. * $p < 0.05$ and ** $p < 0.01$, significant difference as compared to the control group.

barrier function, improved by promoting the production of hyaluronic acid in the dermal fibroblasts, might be one of the causes of the decrease in ear thickness. On the other hand, the FBE and FBEP did not clearly influence serum IgE, IL-4, or INF- γ , reducing only auricle thickness. T-cells do not appear to be the target of the anti-inflammatory action of FBE and FBEP. It has been reported that several plant polyphenols reduce the release of inflammation-inducing substances.²³ Furthermore, it is thought that the anti-inflammatory effect of genistein in NC/Nga mice is due to suppression of chemical mediators released from mast cells as a key effector cell in early-phase allergic inflammation,²⁴ and a similar effect can be also expected for the barley polyphenols contained in FBE and FBEP.

We examined the influence of combined administration of FBEP and GABA upon AD-like skin lesions but were unable to confirm any additive/synergistic effects on ear thickness reduction. However, only with combined administration of FBEP and GABA was a normalizing effect observed for INF- γ in splenic cells. We confirmed an increase in erythrocyte glutathione peroxidase activity as well as an increased effect on the hepatic GSH level in the mixed feeding test of FBEP using normal mice.⁸ On the other hand, it has been reported that antigen-presenting cells (APCs) contain abundant levels of reduced-type GSH and produce large amounts of IL-12, which can induce differentiation of naive T cells into the Th1 type.^{25,26} The existence of a

positive feedback mechanism has been further confirmed, in which IFN- γ , a Th1 cytokine, increases the ratio of intracellular GSH/GSSG, while IL-4, a Th2 cytokine, decreases this ratio.²⁷⁾ In other words, it is possible that in the case of combined administration of FBEP and GABA, in addition to control of the Th1/Th2 balance by GABA, the effect of FBEP on the intracellular GSH redox state influences the Th1/Th2 balance to produce Th1 dominant status. In order to confirm this, it is necessary to investigate the influence of FBEP on GSH content and production of IL-12 by APC.

We have obtained an interesting finding on the combined administration of FBEP and GABA in a human study. In the open human study, following intake of a drink containing 2,000 mg of FBEP and 150 mg of GABA for 4 weeks continuously, the subjects were confirmed to have significantly higher levels of secretory IgA (s-IgA) in the saliva as compared to the level before intake.²⁸⁾ It has been suggested that s-IgA in the saliva is a major immunological factor in preventing the invasion of microorganisms and macromolecular foreign substances (antigens) into mucosal areas, and that s-IgA in the saliva in a patient exhibiting pollinosis or perennial rhinitis is clearly lower than in a healthy person, and that a low s-IgA level is potentially related to allergic sickening.²⁹⁾

Based on a comparison of the thickness-reducing effect of FBE and FBEP, the anti-inflammatory substances in FBE are thought to have been concentrated to FBEP, but the ingredients related to the anti-inflammatory effects of FBEP are not yet known. Further examination is necessary to identify the ingredients involved in FBEP, including the mechanism of anti-inflammatory action with respect to combined administration of FBEP and GABA.

With respect to body-weight changes during the study period, similar normal body-weight increases, as in the normal control group, were observed in all groups for the three substances tested (FBE, FBEP, GABA). Hence it is suggested that these three substances are very safe materials, even for administration as long as 10 weeks. On the other hand, in the PSL group a significant reduction in the increase in body weight was observed. There were hardly any differences in the amounts of intake among the groups, the suppressed body weight gain is not thought to be related to these administrations. A similar, reduced body weight gain was also confirmed in the report of Hirasawa *et al.*,³⁰⁾ although the administration route was by application.

The present study confirms that FBEP and/or GABA reduced AD-like skin lesions in NC/Nga mice. It is suggested that combined administration of FBEP and GABA causes an increase in serum IgE and splenocyte IL-4 and a decrease in splenocyte IFN- γ , having adjusted the Th1/Th2 balance to a Th1 dominant status. Thus it is to be expected that simultaneous intake of FBEP and GABA alleviates allergic symptoms such as AD.

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