Anti-hyperuricemic Effect of Fermented Barley Extract is Associated with Increased Urinary Uric Acid Excretion

Hideki Hokazono1,2*, Toshiro Omori1 and Kazuhisa Ono2

1 Research Laboratory, Sanwa Shurui Co. Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan
2 Department 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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Effects of a fermented barley extract P (FBEP) on uric acid (UA) metabolism in three hyperuricemia rat models were studied. First, the influence of FBEP on UA biosynthesis was examined using rats hypodermically administrated oxonic acid. Then, the influence of FBEP on dietary purine absorption was examined using rats fed a high dose of inosinic acid. Finally, the influence of FBEP on UA excretion was examined using adenine-administered rats. From the first two models, FBEP was found to be rarely, or not at all, involved in the biosynthesis of UA or absorption of nucleic acid. On the other hand, in the rats loaded with adenine, it was shown that the urinary UA of the high-dose FBEP group (4,000 mg/kg/day) was significantly elevated by 27% compared to the control group. In addition, the UA clearance value in the high-dose FBEP group was increased 2-fold compared to the control group. These results suggest that FBEP reduces serum UA by increasing urinary excretion of UA.

Keywords: fermented barley extract, uric acid, hyperuricemia, urate clearance

Introduction

Gout and hyperuricemia are considered to be lifestyle-related diseases, in which environmental factors, in addition to the hereditary background, are largely associated with their incidences. The incidence of gout in Japan has significantly increased since the 1960s as a result of a westernized diet, with the number of patients estimated to be roughly 600,000. The incidence of hyperuricemia, the underlying disease of gout, has been reported to be approximately 20% of Japanese male adults (Hikita, 2000; Guideline for the management of hyperuricemia and gout, 2002). In the selection of methods and medicines for the treatment of hyperuricemia, it is important to classify the disease types. Hyperuricemia is largely classified into 3 types: the overproduction-type, in which the production of uric acid (UA), such as the biosynthesis or decomposition/catabolism of purine, is increased (Hikita, 2000; Guideline for the management of hyperuricemia and gout, 2002); decreased excretion-type, in which the excretion of UA from the kidneys is lowered (Guideline for the management of hyperuricemia and gout, 2002); and mixed-type, in which both the above mentioned types are observed (Imamura et al., 1994). In Japan, the frequency of hyperuricemia by type is reported as follows: 12% UA overproduction-type, 60% lowered UA excretion-type, and 25% mixed-type, making up 85% in total when combining the latter two groups (Imamura et al., 1994; Nakamura, 1979). Hyperuricemia is also an independent predictive factor of metabolic syndrome (Sui et al., 2008) and is often accompanied by lifestyle-related diseases, such as hypertension, abnormal glucose tolerance, hyperlipemia, obesity, etc. Therefore, for the prevention and treatment of gout and hyperuricemia, it is important to not simply control serum UA levels, but also provide preventative lifestyle advice, such as diet and exercise therapy. Among the options for the prevention and treatment of hyperuricemia, limiting calorie intake as a diet therapy is one of the most popular methods. However, it is not necessarily easy to maintain the strict, limited calorie intake for an extended period of time. For gout and hyperuricemia, in which early detection and treatment are important, it is advisable to control the level of UA, even for individuals with only slightly elevated serum UA (who may not satisfy the criteria for pharmacotherapy) or for mild hyperuricemia. In this con-
text, there is an increasing need for food enabling the control of UA levels. For example, the protein in milk products has been reported to lower serum UA levels by increasing the amount of UA excretion into the urine (Garrel et al., 1991). It has also been reported that several flavonoids, which have an inhibitory effect on xanthine oxidase (XOD) activity, are able to reduce serum UA levels by inhibiting UA biosynthesis (Wang et al., 2004; Mo et al., 2007). In addition, several dietary fibers have been reported to reduce the increase of serum UA by inhibiting absorption of dietary nucleic acid (Koguchi et al., 2002; Koguchi et al., 2004).

In FBE obtained from shochu distillery by-products (SDB), physiological effects, such as the inhibitory action on hepatic disorder (Mochizuki et al., 2001; Mochizuki et al., 2005), anti-oxidative stress (Hokazono et al., 2009), and anti-inflammatory action (Giriwono et al., 2010), have been confirmed at the level of animal studies. Furthermore, serum UA levels were significantly reduced after continuous intake of FBEP (obtained from FBE) for 12 weeks in a double blind, parallel group comparison test using Japanese adults with slightly elevated UA levels (Hokazono et al., 2010). The FBEP dose in the clinical study was approximately 2,000 mg/day (dry weight basis), and its components consisted of a wide range of proteins, polyphenols, and dietary fibers. As a result, the components involved in reducing serum UA levels, as well as their detailed mechanism of action, have not yet been clarified.

Thus, the present study aimed to investigate the mechanism involved in lowering human serum UA by FBEP treatment, and to establish an in vivo screening model of the components involved, using three hyperuricemia rat models.

**Materials and Methods**

**Experimental design** This study was composed of three separate experiments. We first examined the effect of FBEP on rats with oxonic acid-induced hyperuricemia (Exp. 1). Next, we investigated the effect of FBEP given to rats with high nucleic acid feed-induced hyperuricemia (Exp. 2). Finally, we investigated the influence of FBEP on adenine-induced hyperuricemic rats (Exp. 3). The animal experiments were performed in accordance with the “Standards Relating to the Care and Management, etc. of Experimental Animals (Notification No. 6, March 27, 1980, of the Prime Minister’s Office, Japan, revised May 28, 2002)” and “Japan’s Act on Welfare and Management of Animals (up to the revisions of Act No. 50 of 2006)”.

**Materials** Allopurinol, potassium oxonate and adenine were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Inosinic acid was purchased from Kyowa Hakko Kogyo, Inc. (Tokyo, Japan). Water for injections and sodium carboxymethyl cellulose (CMC) were purchased from Otsuka Pharmaceutical Factory, Inc. (Tokushima), and Junsei Chemical, Inc. (Tokyo, Japan), respectively. All other reagents were purchased from Wako Chemical, Inc. (Tokyo, Japan). The preparative procedure of FBE and FBEP are as follows:

Fresh barley-SDB separated during vacuum distillation, provided by Sanwa Shurui Co. Ltd. (Oita, Japan), was prepared using pearled barley (PB; barley pearled approximately 65% of its initial weight) as the raw material. 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, named fermented barley extract (FBE), was concentrated by vacuum evaporation. FBE concentration was measured as Brix values by a portable refractometer (PAL-1; Atago, Tokyo, Japan). Brix 8 FBE obtained from barley-SDB was concentrated to Brix 60.

A 10-L volume of FBE before concentration was subjected to an Amberlite FPX66™ (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, named FBEP, was obtained from 10 kg of PB. The recovery rates of FBE and FBEP from PB were 10% and 1% on a dry weight basis, respectively. The nutritional components of the samples were analyzed and are shown in Table 1.

**Animals** Four-week-old male SD rats for Exp. 1, and 5-week- and 4-week-old male Wistar rats (SLC Japan Co., Ltd., Shizuoka, Japan) for Exp. 2 and 3, respectively, were

<table>
<thead>
<tr>
<th>Components</th>
<th>FBE</th>
<th>FBEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>43.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>23.6</td>
<td>39.2</td>
</tr>
<tr>
<td>Crude fat</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Ash</td>
<td>3.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>1.0</td>
<td>8.8</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>0.9</td>
<td>9.2</td>
</tr>
</tbody>
</table>

FBE, Fermented barley extract; FBEP, Fermented barley extract P.
Effects of Fermented Barley Extract on Hyperuricemic Rats

subjected to experimentation after a one-week period of quarantine and domestication. In order to make the average body weight and standard deviations almost equal among the groups, the rats were classified into the following groups: For Exp. 1 and 2, each group consisted of 8 rats, and for Exp. 3, the untreated group consisted of 6 rats and the other groups consisted of 10 rats. Individual identification of the rats was accomplished by applying picric acid to the body hair. Rats were housed 2 per stainless steel cage and the following conditions were set for the animal room: temperature, 21-27°C; relative humidity, 40-70%; ventilation, 18 times/h; and lighting, 12 h (7:00 to 19:00). The rats were allowed food and water ad libitum.

Diet In Exp. 1, the rats were fed a MF solid diet (Oriental Yeast, Inc., Tokyo, Japan). In Exp. 2, the rats were fed either a MF-powdered basal diet or a high nucleic acid diet, with 20% inosinic acid added to the basal diet. In Exp. 3, the rats were fed a MF-powdered basal diet and received no treatment, or received a diet containing 0.75% adenine.

Analytical procedures In experiment 1, the effect of FBEP on UA biosynthesis was investigated.

1) Group configuration: The experiment consisted of five groups: a control group, three FBEP administration groups (dose: 1700, 3400, or 6800 mg/kg), and an allopurinol administration group (dose: 10 mg/kg).
2) Administration method: Injection water, FBEP suspensions, and an allopurinol suspension were given to the control, FBEP, and allopurinol groups every day from day 0 to day 14, respectively. The fluids (10 mL/kg each) were given by forced oral administration using a sonde tube for rats.
3) Hyperuricemia induction method: Rats were fed a MF diet containing 20% inosinic acid ad libitum between days 7-14.
4) Body weight measurement: Individual body weights were measured at days 0, 4, 7, 11 and 14.
5) Food consumption: The amounts of residual feed and provided feed were measured on the day of body weight measurement to calculate the daily food intake per individual for each cage.
6) Urine volume and pH: The rats from each group, except the untreated group, were transferred to metabolism cages on the evening of day 13 until the morning of day 14 for urine collection, and the volume of collected urine was measured using a measuring pipette. All the rats of the untreated group were transferred to metabolism cages on the evening of day 17 until the morning of day 18 for urine collection, and urine volumes were measured as above. Fresh urine from day 13 was used for pH measurement with a test paper (Uropaper III Eiken, Eiken Chemical Co., Ltd., Tokyo, Japan).
7) Serum UA: On day 14, rats were anesthetized with diethyl ether prior to collection of blood samples from the caudal artery for serum isolation. An aliquot of 200 μL of serum was mixed with 25 μL 4 mol/L perchloric acid. The mixture was then mixed with 20 μL 4 mol/L potassium dihydrogen phosphate, centrifuged (20,000 × g, room temperature, 10 min), and the supernatant was collected for UA measurement. UA concentration was measured by HPLC: column, Sim-Pack CLC-NH2 (ϕ 6.0 mm × 150 mm, Shimazu Co., Ltd., Kyoto, Japan); mobile phase, CH3CN/20 mM KH2PO4 = 3/2 (v/v).
8) Serum and urinary allantoin: Serum and urine samples taken on day 14 were measured for allantoin concentration by HPLC: column, Atlantis HILIC Silica (ϕ 4.6 mm × 150 mm, Waters, Milford, MA, USA); mobile phase, CH3CN/H2O = 95/5 (v/v). The serum and urine samples were diluted two-fold with distilled water and centrifuged (20,000 × g, room temperature, 10 min) to obtain supernatants, which were then passed through an ultrafiltration membrane (Microcon Ultracel YM-10 Regenerated Cellulose 10,000 MWCO, Millipore, Bedford, MA, USA). Three volumes of CH3CN were added to each ultrafiltrate and the mixtures were centrifuged (20,000 × g, room temperature, 10 min) to obtain supernatants for HPLC.

In experiment 3, the effect of FBE and FBEP on UA excretion was investigated.

1) Group configuration: The experiment consisted of five
groups: an untreated group, control group, two FBEP administration groups (dose: 2,000 or 4,000 mg/kg), and the FBE administration group (dose: 6,500 mg/kg).

2) Administration method: The untreated and control groups were administered water injections, FBEP and FBE groups were administered each soil suspension from day 0-14. The administered volume of the fluids was 10 mL/kg each time. An oral sonde for rats was employed for forced administration twice a day.

3) Hyperuricemia induction method: Adenine was mixed with the feed at a ratio of 0.75%, which was freely accessible from day 0 to day 14.

4) Body weight measurement: Individual body weights were measured at days 0, 4, 7, 11 and 14.

5) Food consumption and water intake: The amounts of residual food and water, and the amounts of provided food and water, were measured on the day of body weight measurement to calculate daily intake of food and water per individual.

6) Urine volume: All rats were transferred to metabolism cages at 10:00 am on day 14 to collect urine for 6 h up to 16:00. The volume of the collected urine was measured with a measuring pipette. Rats transferred to metabolism cages were deprived of water and feed.

7) Urine pH: Ames urine test paper (Siemens Medical Solutions Diagnostics, Inc., Tokyo, Japan) was used to measure the pH of fresh urine on day 14.

8) Serum or urinary UA, allantoin, creatinine and urea nitrogen: Rats were anesthetized with diethyl ether on day 15 to take blood samples from the ventral aorta, and the blood samples were centrifuged to obtain serum samples. The urine samples consisted of supernatants prepared by centrifuging urine collected on day 14. The urine samples were diluted with 3 volumes of distilled water for the measurement of urinary UA concentration. Five volumes of CH3CN were added to each of the urine samples and the mixtures were centrifuged (16,000× g, room temperature, 10 min) and the supernatants were used for the measurement of urinary allantoin concentration. UA and allantoin concentrations of the sera and urine were measured by the same methods as used in Exp. 2. Urea nitrogen concentrations of the sera and urine were measured using a B-Test (Wako) for urea nitrogen, and allantoin concentrations of the sera and urine were measured using a Creatinine-Test (Wako).

9) UA clearance: Based on measurements of serum UA concentration, urinary UA concentration, and urine volume, the following equation was used to calculate UA clearance (Clur).

\[
Cl_{ur} (mL/kg/min) = \frac{U_{ur} (mg/mL) \times V (mL/kg/min)}{P_{ur} (mg/mL)}
\]

- \( P_{ur} \): Serum UA (mg/mL)
- \( U_{ur} \): Urinary UA (mg/mL)
- \( V \): Urine volume/kg body weight/min (mL/kg/min)

Statistical Analyses

Dunnett’s test, U test, and Kruskal-Wallis test were used to test statistically significant differences between the control group and the test groups. For Exp. 2 and 3, Student’s \( t \)-test and U test were used to test statistically significant differences between the control group and the untreated group. A significance level of 5% was adopted.

Results

Exp. 1 Although the data is not given in the tables, the average body weight on days 0, 4, and 7 were approximately 140 g, 170 g, and 200 g, respectively. There were no significant differences among the groups. Table 2 shows serum UA concentration. The FBEP groups tended to be lower in serum UA concentration than the control group, but no significant differences were observed. In contrast, serum UA concentration was significantly lower in the allopurinol group, a positive control, than in the control group (\( p < 0.001 \)).

Exp. 2 Body weight gain was -31% in the high-dose FBEP group and -86% in the allopurinol group when compared with the control group (\( p < 0.05 \) and \( p < 0.001 \), respec-

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>FBEP (LD)</th>
<th>FBEP (MD)</th>
<th>FBEP (HD)</th>
<th>Allopurinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>5.3 ± 0.7</td>
<td>5.1 ± 0.6</td>
<td>4.5 ± 0.6</td>
<td>4.7 ± 0.7</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>P value (vs control)</td>
<td>—</td>
<td>NS</td>
<td>0.059</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

FBEP, fermented barley extract P; LD, low-dose; MD, medium-dose; HD, high-dose.

Values are expressed as means ± SD.

The \( p \)-values were calculated according to Dunnett’s test.
Food consumption was -13% in the high-dose FBEP group and -32% in the allopurinol group when compared with the control group (p<0.05 and p<0.01, respectively).

Table 3 gives the serum UA and serum allantoin concentrations. The serum UA concentration was significantly higher in the control group than in the untreated group (p<0.001), indicating that the high-inosinic acid feed induced hyperuricemia. Serum UA was significantly lower in the allopurinol group than in the control group (p<0.001), but there were no significant differences between the FBEP groups and the control group. As was the case with the serum UA concentration, the serum allantoin concentration was significantly lower in the untreated and allopurinol groups than in the control group. In contrast, there were no significant differences between the FBEP groups and the control group.

Urine volume was significantly smaller in the untreated group than in the control group (p<0.001). However, there were no significant differences in urine volume among the groups given the high-inosinic acid feed. Urine pH was significantly higher in the allopurinol group than in the control group (p<0.01). Urinary allantoin concentration was significantly higher in the control group than in the untreated group (p<0.001). In contrast, there were no significant differences among the groups given the high-inosinic acid feed.

Figure 1 shows the amount of allantoin excreted into the urine per 100 g body weight. The amount of urinary allantoin excretion was significantly higher in the high-dose FBEP group than in the control group (p<0.05).

Exp. 3

Body weight gain, food consumption, and water intake of rats fed an adenine diet are shown in Table 4. As expected, the untreated group increased in body weight during the experiment. In contrast, all adenine groups, including the control group, showed either an intense suppression in body weight increase or body weight decrease, which is believed to have resulted from adenine administration. However, the high-dose FBEP and FBE groups had significantly higher weight gains than the control group (p<0.05). The amount of food consumption by all the adenine groups, including the control group, was small throughout the administration period. The amount of food consumption by the high-dose FBEP group was slightly elevated compared with the control group. Water intake by the control group was significantly higher than the untreated group (p<0.001). The high-dose FBEP and FBE groups consumed significantly more water than the control group (p<0.01 and p<0.05, respectively).

Urine volume and pH of rats fed an adenine diet are shown in Table 5. Urinary volume of the control group showed the tendency to be high as compared with the untreated group (p=0.054). Urinary volume was significantly

### Table 3. Serum uric acid and allantoin concentrations of rats fed a high nucleic acid diet (Exp. 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>Untreated</th>
<th>Control</th>
<th>FBEP (LD)</th>
<th>FBEP (HD)</th>
<th>Allopurinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (mg/dL)</td>
<td>0.5 ± 0.1***</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.3 ± 0.2***</td>
</tr>
<tr>
<td>Allantoin (mg/dL)</td>
<td>1.7 ± 0.1***</td>
<td>16.5 ± 3.9</td>
<td>17.4 ± 3.9</td>
<td>20.6 ± 4.8</td>
<td>10.3 ± 5.0*</td>
</tr>
</tbody>
</table>

FBEP, fermented barley extract P; LD, low-dose; HD, high-dose.

Values are expressed as means ± SD.

Significantly different from the value of rats fed a control diet, *p<0.05, ***p<0.001.
higher in the high-dose FBEP and FBE groups than in the control group (p<0.01 and p<0.05, respectively). Urinary pH was significantly lower in the control group than in the untreated group (p<0.001). There were no significant differences in urinary pH between the control group and other test groups.

Serum or urinary UA, allantoin, creatinine and urea nitrogen are shown in Table 6. There was no significant difference in serum UA concentration between the control group and the untreated group. The FBEP group showed no significant difference in serum UA concentration from the control group; however, the FBE group was significantly lower (p<0.05). The control group was significantly higher than the untreated group in serum allantoin, creatinine, and urea nitrogen concentrations (p<0.01). On the other hand, none of the groups administered the test materials showed a significant difference from the control group. The control group was significantly lower in urinary UA concentration than the untreated group (p<0.01). The high-dose FBEP group was significantly higher in urinary UA concentration than the control group (p<0.05). The control group tended to be lower in urinary allantoin concentration than the untreated group (p=0.0951), and none of the test groups showed a significant difference from the control group. The control group was significantly lower in urinary creatinine and urea nitrogen concentrations than the untreated group (p<0.01). None of the test groups showed a significant difference from the control group.

Figure 2 shows Clur values. The control group was significantly lower in Clur than the untreated group (p<0.01). Only the high-dose FBEP group was higher in Clur than the control group (p<0.01).

Discussion

The process of metabolizing intracellular purines such as adenosine and guanosine, and that of decomposing xanthine into UA, are found to exist in all living things. However, some groups of enzymes involved in decomposing UA into...
substances more water-soluble may have been partially inactivated. Therefore, the final metabolites from a purine metabolizing process may differ by species. In Anthropoidea, including humans, uricase is inactivated and the final metabolite from purine metabolism is UA (Keilin, 1959; Yeldandi et al., 1996; Moriwaki et al., 1999; Hayashi et al., 2000). On the other hand, in rodents such as mice and rats, which belong to the same polyphagia as humans and are frequently used in animal tests, UA is metabolized by uricase, a UA-oxidizing enzyme, into allantoin and excreted in urine (Stavric et al., 1978). Accordingly, we prepared hyperuricemia model-rats by administering the uricase inhibitor oxonic acid (Exp. 1), and examined the effect of FBEP on reducing serum UA levels. Since the serum UA value of five-week-old male SD rats is 0.49-0.74 mg/dL (Yoshizumi et al., 2005), it is thought that serum UA of the control group increased approximately 10-fold compared to untreated rats. As a result, the group administered the potent UA production inhibitor allopurinol exhibited a remarkable reduction in serum UA levels; whereas, similar reductions in serum UA levels were not observed in the group administered FBEP. Therefore, the effects of FBEP on serum UA levels observed in humans was not reproduced in this animal model. In addition, as a result of the in vitro examination on the effect of FBEP upon XOD,
a metabolic enzyme for purine, no inhibitory effect on XOD was observed at concentrations below 1000 μg/mL (data not shown). Consequently, it is considered that FBEP may not substantially inhibit the biosynthesis of UA.

In Exp. 2, we examined the effect of FBEP using rats administered a high dose of nucleic acid. As a consequence, in comparison with the control group, none of the FBEP-dose groups showed a statistically significant, lowering effect on serum UA concentrations. However, for the group administered a high dose of FBEP, the body-weight converted values of the excreted amount of allantoin in urine showed significantly higher values as compared to the control group. A dose-dependent relationship between FBEP administration and UA excretion was not observed. It is thought that the lowest dosage in which a UA excretion promotion effect is observed was the administration level of the FBEP high-dose group. We consider that a dose-finding study is required in the future. The elevated serum and urine allantoin concentrations indicated that the metabolic amount of nucleic acid increased due to the feed containing a high dose of inosinic acid, but subsequently UA metabolism occurred via a metabolic route not existing in humans. In other words, FBEP may not inhibit absorption of nucleic acid in the digestive tract.

Based on the results of Exp. 1 and 2, the mechanism of FBEP in lowering serum UA levels may be the promotion of UA excretion in urine. Thus, for Exp. 3, we designed a study using rats fed an adenine diet, with the hyperuricemia characterized as the decreased excretion-type (Yokozawa et al., 1982; Yokozawa et al., 1983). As a result, the serum UA levels in the group administered FBE, as a sample of FBEP in the pre-purification stage, showed significantly lower values compared with the control group, while the high-dose FBEP group, although the effect was much weaker than in the FBE group, showed a lowering tendency compared with the control group. In the analysis of the UA concentration in urine, the FBEP high-dose group showed significantly high values compared with the control group. A possible reason why serum UA did not decrease significantly in the FBEP high-dose group is that there may have been differences in the metabolism of UA to allantoin. That is, the serum allantoin of FBEP high-dose group is the lowest. In addition, it has been reported that the serum allantoin level of rats fed an adenine diet was remarkable increased, whereas the serum UA level did not change considerably (Yokozawa et al., 1981). Yokozawa et al. consider that the excessive UA was metabolized to allantoin in order not to raise UA levels in the body beyond a certain level. Although Exp. 3 was used as a decreasing excretion model of UA, it may be applicable as a model of lowered UA excretion-type hyperuricemia by inhibiting uricase. Furthermore, as the FBEP high-dose group showed significantly elevated levels in both water intake and urine production compared with the control group, FBEP appears to be able to further reduce elevated serum UA concentrations by excreting a comparatively large amount of urine containing relatively high UA levels. In addition, by computing and comparing Clur, the action by which FBEP improved UA excretion ability, which declined by adenine diet, became clearer. From the UA clearance data, it is thought that the uricosuric promotion component in FBE was concentrated to FBEP. In a 24-hour urine collection in the clinical trial, changes in urinary volume were not observed, but increasing urinary UA concentration and UA clearance were observed by 12 weeks of FBEP treatment (Hokazono et al., 2010). Because an increase in UA clearance was observed in human and rat with a depression of UA clearance, it is suggested that FBEP improved UA metabolism by promoting the excretion of UA. On the other hand, FBEP or FBE treatments were unable to significantly alter the effect of adenine administration on indicators of renal disorders (serum urea nitrogen and creatine concentrations) and severe renal toxicity was confirmed in all adenine-administered groups.

As characteristic components contained in FBEP, the secondary metabolites of Aspergillus species that possess antioxidant activity have been reported (Miyake et al., 2007). On the other hand, the intake of vitamin C, a water-soluble antioxidant, reduces serum UA levels (Huang et al., 2005). Moreover, higher vitamin C intake is independently associated with a lower risk of gout (Choi et al., 2009). As mechanisms by which vitamin C reduced serum UA, increase in glomerular filtration and/or competition for renal reabsorption are considered. The mechanism by which FBEP reduced serum UA may have been through the inhibition of renal re-absorption of UA, by a substance such as vitamin C, which is reabsorbed via anion-exchange transport at proximal tubules. Furthermore, it has been reported that the consumption of polyphenol-containing cherries in healthy women increased urinary UA excretion and decreased plasma UA (Jacob et al., 2003).

On the other hand, it has been reported that an intake of milk and soy-originating protein increased urinary UA excretion and clearance (Garrel et al., 1991). Furthermore, it has been reported that urinary UA excretion increased by the intravenous administration of amino acids (Yamamoto et al., 1999). That is, it is necessary to examine the influence of anionic peptides contained in FBEP on UA excretion.

The mechanism involved in the promoting action of FBEP on UA excretion requires further study, and it will be necessary to investigate what effect FBEP may have upon the UA transporter responsible for the re-absorption of UA.
from renal tubules, such as urate transporter 1 (Enomoto et al., 2002).

The present findings suggest that the mechanism involved in the lowering effect of FBEP on serum UA levels may be attributed to the promotion of urinary UA excretion. In conclusion, it is thought that the adenine-loaded rat model, producing lowered UA excretion-type hyperuricemia, is the most appropriate in vivo screening model for examining the active components in FBEP and their involvement in lowering serum UA levels in humans.

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