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Protective Effects of Barley and Its Hydrolysates on Gastric Stress Ulcer in Rats

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This research intends to clarify the protective effect of barley and its hydrolysates with respect to a water immersion stress-induced ulcer in the rat model. The β-(1→3)-glucan content of barley, and specifically β-(1→4),(1→3)-glucan content was determined and then gastric stress ulcerogenesis induced by water immersion was conducted using five-week-old male Sprague-Dawley rats (7 rats in one group). The barley diet group was fed 10% barley flour that was substituted with sucrose in the control diet. For the 3 groups fed on soluble dietary fiber (SDF), the diets were supplemented with 0.46 g of SDF, equivalent to 100 g of the control diet; 0.46 g of SDF is equivalent to 10 g of barley flour. The rats were housed in a stress-cage and immersed in a water bath (23°C) up to their necks for 21 h. The content of SDF and β-(1→3)-glucan content in barley flour were 4.6% and 3.4%, respectively. Although strongly anti-ulcer activities were observed in the barley (10%), SDF isolated and β-(1→3)-glucan fraction (Hydrolysate I) prepared from barley flour after treatment with lichenase, in other words, β-(1→4),(1→3)-glucan itself, its hydrolysate (Hydrolysate II) with β-(1→3)-glucosidase did not display any anti-ulcer activity. This finding suggests that the β-(1→3)-glucosyl-linkage on β-(1→3)-glucan is an important part of the active principle for anti-ulcerogenesis.

Key words——β-(1→3)-glucan; gastric stress ulcer; barley; dietary fiber; rat

INTRODUCTION

Today, barley is used mostly as alcoholic material for beer and whisky or feed for animals, whereas use for human consumption is quite limited. Barley is recognized internationally as an important cereal, although it has not been featured in the scientific literature with the same prominence as some other grains especially such as rice and wheat. Interest in barley as a food source is currently resurging because of its potential to contribute dietary fiber, and especially water soluble dietary fiber (SDF) β-(1→3)-glucan, as well as its nutritional value and minor components like vitamins and minerals. Conventionally, dietary fiber is considered to have no dietary value as it is not absorbed in the body. However, dietary fiber has attracted attention recently due to its diverse physiological significance. In particular, the role of β-(1→3)-glucan, a SDF, as an anti-inflammatory activity has been reported.[1—3] β-(1→3)-Glucan is known to be a polysaccharide found in algae, mushrooms, yeast, and higher plants.[4] The authors have been investigating barley containing β-(1→3)-glucan for human consumption.[5] Previously, a water immersion restraint rat model[6] has been used for the study of stress gastric ulcer. The stress ulcer caused by water immersion restraint is a type of acute gastric mucosal damage. Furthermore, a similar petechial hemorrhage found in the early stage of human chronic gastric ulcer has also been seen in the stomach of water immersion restraint rats.

The authors investigated the chronological effect of barley (10%) diet on gastric juice secretion (serum gastrin concentration, gastric juice acidity and pepsin activity) during ulcerogenesis induced by water immersion stress.[7] These results indicate that suppression of the water immersion induced stress ulcer is partially caused by decrease in the aggressive factors such as acid and pepsin. The present paper attempts to compare the protective effect of β-(1→4), (1→3)-glucan from barley flour on gastric stress ulcer induced by water immersion in rats.

MATERIALS AND METHODS

Prosky and McCleary Method SDF samples were isolated from barley flour by the method of Prosky.[8] Each sample was treated with 3 types of enzymes, termamyl 120L (Wako Pure Chemical Indus-

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tries, Ltd., Osaka, Japan), protease and amyloglucosidase (Sigma Chemical, St. Louis, MO, USA), to digest starch and protein. After centrifugation (1,100×g, 10 min, high-speed refrigerated centrifuge 7800, Kubota, Tokyo, Japan) to eliminate insoluble dietary fiber (IDF), SDF was obtained by 95% ethanol precipitation.

β-(1→3)-Glucan content, and specifically β-(1→4),(1→3)-glucan content, was determined by the McCleary method[9,10] using lichenase (Megazyme International, Bray, Ireland) to hydrolyze the 1,4-linkage adjoining the 1,3-linkage and liberated various oligosaccharides and also using β-(1→3)-glucosidase (Megazyme) to cleave the 1,3-linkage and to release glucose. The glucose released was determined by the Glucose B-test Wako (Wako) at an absorbency of 505 nm. β-(1→3)-Glucan content was calculated from the amount of glucose released.

Composition of the Experimental Food With the development of technology for barley processing using a milling machine, HS-250L (Chiyoda Engineering Co. Ltd., Tokyo, Japan), each fraction of barley flour classified into grain and flour was continuously and stably obtained at a highly uniform quality and shape without high external temperature and humidity.[11,12]

The barley flour was prepared with a 75% yield to feed the rats. Then the barley flour thus milled was filtered through 100-mesh at the time of preparation. As shown in Table 1, the same control diet (Oriental Yeast, Co., Ltd., Tokyo, Japan) as in the previous report was used.[7] The control diet contained no SDF. The barley diet group was fed 10% barley flour that was substituted with sucrose in the control diet. As shown in Scheme 1, SDF was treated with lichenase to cleave the 1,4-linkage adjoining the 1,3-linkage and its hydrolysate was treated with β-(1→3)-glucosidase. For the 3 groups fed on SDF, the diets were supplemented with 0.46 g of SDF, equivalent to 100 g of the control diet; 0.46 g of SDF is equivalent to 10 g of barley flour as shown later.

Experimental Animals Five-week-old male Sprague-Dawley rats weighing 110−130 g (Clea Japan, Inc., Tokyo, Japan) were housed in individual cages in an animal room with a 12 h dark: light cycle (lights on from 8：00—20：00) at 23±1℃ and 55±5% humidity. All groups were given the control diet for two days prior to experiments, and then the

Sample 500 mg
- Ethanol aqueous solution 50%, 1.0 ml
- Distilled water (pH 6.8), 5.0 ml
- Stir using a Vortex mixer
- Heat in boiling water bath
- Stir using a Vortex mixer
- Cool, 40℃
- Lichenase (10 U) in distilled water
- Stir using a Vortex mixer
- Incubate, 40℃, 1 hr

Hydrolysate I
- β-glucosidase (5.5 U)
- Stir using a Vortex mixer
- Incubate, 40℃, 15 min

Hydrolysate II

Scheme 1. Preparation of SDF Hydrolysate Using Lichenase and β-(1→3)-Glucosidase

Table 1. Composition of the Experimental Diets (%)

	Control	Barley (10%)	SDF isolates	Hydrolysate I	Hydrolysate II
Casein	20	20	20	20	20
Oil*	5	5	5	5	5
Minerals**	5	5	5	5	5
Vitamins**	2	2	2	2	2
α-Starch***	12	12	12	12	12
β-Starch***	28	28	28	28	28
Sucrose	28	18	28	28	28
Barley****	—	10	—	—	—
SDF	—	—	0.46	0.46	0.46

* Oil: Soybean oil: fish liver oil, 4：1. ** Harper mixture[13]. *** α,β-Starch: Corn starch. **** Barley: Draft classified barley flour. Hydrolysate I was treated with lichenase to cleave the 1,4-linkage adjoining the 1,3-linkage. Hydrolysate II was treated with β-(1→3)-glucosidase to hydrolysate I. SDF: Soluble dietary fiber.

barley (10%) diet group and 3 groups fed on SDF were switched to the barley (10%) diet and SDF sample diets during the study. The experimental diets were prepared by mixing 10 g of the diet with 6 ml of tap water evenly to make a paste before administration to the rats and the rats were allowed free access to the experimental diets and tap water for 14 days. The diet intake and body weight during the experiment were recorded every day.

Water Immersion Induced Stress Ulcer Tests The stress experiment was done according to the method developed by Takagi and Okabe.[6] The rats were given only water but no food for 24 h after the 14-day experimental period. Subsequently, the rats were housed in a stress-cage and immersed in a water bath (23℃) up to their necks. The rats were given neither food nor drink during the stress test. To perform the investigation, the water immersion stress was sustained for 21 h.

After water immersion stress, the rats were anesthetized with diethyl ether. After making an abdominal incision, the stomach was surgically removed immediately from the rats. The isolated stomach was washed to remove blood, and the ulcer index and protective rate were measured and calculated in the same manner as reported[14] after fixation with 10% formalin. The care and treatment of the experimental animals conformed to the guidelines of Koshien University for the ethical treatment of laboratory animals.

Statistical Analysis Data are presented as the mean±standard deviation. Differences among experimental group were evaluated by Tukey's multiple test[15] following one-way analysis of variance (ANOVA) and considered significant at $p<0.05$. The statistical analysis program used in this study was SPSS (Ver.11.0J, SPSS Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

Yields and Purity of SDF SDF of barley is obtained by the method of Prosky with a yield of 4.6%. The purity of β-(1→4),(1→3)-glucan was 21.3% as shown in Table 2. These isolates were used in the animal feeding tests as SDF samples. The barley is a Japanese normal cultivar of nonglutinous barley. In general, the content of β-(1→3)-glucan was higher in the grains of glutinous barley strains than nonglutinous ones. The β-glucan content of high amylose barley is reported to exceed 7%.[16]

Growth and Food Intake of Experimental Groups The food intake and body weight gain during the study are shown in Table 3. No significant difference in food intake was observed between the five groups. Additionally, the rats in the five groups grew healthily and no significant difference in body weight gain was observed in the five groups. Delaney et al.[17] reported evaluation of the toxicity of β-glucan-enriched soluble fiber from barley in rats with dietary administration at concentrations of 0.7, 3.5, and 7% β-glucan for 28 days. Results of this study indicated that the consumption of concentrated barley β-glucan was not associated with any obvious signs of toxicity in rats even following consumption of large quantities. These results suggest that the barley (10%) diet has no effect on the growth and food intake of rats.

Table 2. Yields and Purity of β-(1→4),(1→3)-Glucan Isolates

Sample	Yields of isolate (%)	Purity (%)	Yields of β-glucan (%)[‡]
Draft barley	100	3.4	3.40
Isolate (SDF)[‡‡]	4.6	21.3	0.99

[‡] Yields of β-glucan were determined by McCleary method. [‡‡] SDF samples were isolated from barley flour by method of Prosky.

Table 3. Food Intake, SDF Intake and Body Weight Gain (g/14 Days)

Diet	Food intake[###]	SDF intake	Body weight gain[###]
Control	321±8	—	111±10
Barley (10%)	310±11	1.4	120±12
SDF isolates	325±9	1.5	121±13
Hydrolysate I	292±13	1.4	107±9
Hydrolysate II	352±17	1.6	127±12

[###] Values are mean±S.D. for 7 rats. For abbreviations, *see* the legend for Table 1.

Effects of Barley and Its Hydrolysates on Gastric Stress Ulcer The ulcer index and ulcer protective rates after the water immersion stress are shown in Table 4. Generally, in the water immersion stress-induced ulcer model, punctuated or linear erosions are observed only in the gastric mucosa,[18] and the same finding was obtained in the present experiments. Additionally, none of the groups displayed stigmatism in the stomach prior to water immersion stress.

Although strongly anti-ulcer activities were observed in the barley (10%), SDF isolated and β-(1→3)-glucan fraction (Hydrolysate I) prepared

Table 4. Effect of Barley (10%) Diet and SDF Diets on Water Immersion Induced Stress Ulcer in the Rats

Diet	Ulcer index (mm^2) mean±S.D.###	Protective rate (%) Control
Control	52.9±6.4[a)]	—
Barley (10%)	26.3±11.8[b)]	50.3
SDF isolates	19.4±5.1[b)]	63.3
Hydrolysate I	13.5±4.7[b)]	74.5
Hydrolysate II	56.8±3.9[a)]	−7.4

Values are mean±S.D. for 7 rats. For abbreviations, *see* the legend for Table 1. *a*),*b*) Means not sharing a common superscript letter differ significantly in Tukey's multiple test ($p<0.05$).

from barley flour after treatment with lichenase, in other words, β-(1→4),(1→3)-glucan itself, its hydrolysate (Hydrolysate II) with β-(1→3)-glucosidase did not display any anti-ulcer activity as shown in Table 4. This suggests that β-(1→3)-glucosyl-linkage on β-(1→3)-glucan plays an important role in anti-ulcerogenesis. Ohtake et al.[19] reported on the anti-ulcer activity of young barley leaves. Results of this study indicated that a fraction containing a water-soluble organic compound displayed significant anti-ulcer activity in the stress-induced gastric ulcer.[19] This fraction did not affect the secretion of acid or pepsin from the stomach in rats.[19] The authors also reported the effect of barley on gastric juice secretion (serum gastrin concentration, gastric juice acidity and pepsin activity) during ulcerogenesis induced by water immersion stress.[7] The serum gastrin concentration was significantly lowered, the suppressive effect on the gastric juice acidity was observed 4 h after the start of the stress experiment.[7] In the addition, the pepsin activity was significantly decrease at 2 h, indicating the suppressive effect of the barley contained in the diet on the ulcerogenesis.[7] These results indicate that the suppression of the water immersion induced stress ulcer is partially caused by the decrease in the aggressive factors such as acids and pepsin. However, the involvement of protective factors can not be excluded as the decrease in the aggressive factors took place for short duration.

Two fraction peaks were obtained from the hydrolysate of the SDF sample by lichenase using Biogel P-30 chromatography. Although the 1st peak did not contain β-glucan, the 2nd peak which contained β-(1→4),(1→3)-glucan, was important. This peak displayed a molecular weight of around 23,000 in comparison to molecular weight markers.[20] Further investigation is required to reveal the relationship between the suppressive effect of β-(1→3)-glucan on water immersion stress-induced ulcer and protective factors.

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