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Hepatoprotective Effects of Potato Peptide against D-Galactosamine-induced Liver Injury in Rats

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Abstract The effect of some peptides on hepatoprotection and cecal fermentation against D-galactosamine (GalN)-treated rats was studied. In acute hepatic injury tests, serum alanine aminotransferase (ALT), aspartate aminotranferase (AST), and lactic dehydrogenase (LDH) activities were remarkably increased after injection of GalN. However, potato and soybean peptides significantly decreased GalN-induced alterations of serum ALT and AST activities. Hepatic thiobarbituric acid-reactive substance (TBARS) concentration in GalN-treated groups fed potato and soybean peptides was significantly lower than that in GalN-treated control group. Hepatic glutathione level in the GalN-treated group fed potato peptide was significantly higher than that in GalN-treated control group. Furthermore, cecal *Lactobacillus* level in GalN-treated groups fed potato and soybean peptides was significantly higher than that in GalN-treated control group, and cecal short-chain fatty acid concentrations in GalN-treated group fed potato peptide were significantly higher than in GalN-treated control group. These results indicate that potato peptide may improve the cecal fermentation and prevent the GalN-induced liver damage in rats.

Keywords: potato peptide, D-galactosamine, cecal flora, short-chain fatty acids, hepatoprotection

Introduction

Liver damage occurs via direct injurious attack by a wide variety of primary hepatotoxins, like as alcohol, aflatoxin, heavy metals, and drugs. Of them D-galactosamine (GalN) is similar to human viral hepatitis in its morphological and functional features (1). It has been reported that GalN induces liver injury by inhibiting the synthesis of RNA and protein through a decrease in the cellular uridine triphosphate (UTP) concentration (1,2). Furthermore, it has been reported that GalN-induced-apoptosis and necrosis are connected in a free radical-dependant fashion in primary culture of rat hepatocytes (3). Recently, several researchers have reported that natural antioxidants and components of plant origin may prevent free radical-mediated liver damage *in vivo* and *in vitro* (4-6).

Bacterial fermentation of complex dietary carbohydrates by the large bowel microflora has attracted considerable interest because the resulting short-chain fatty acids (SCFA) have potentially beneficial effects on large bowel physiology (7). Major acids (acetate, propionate, and butyrate) are absorbed and metabolized rapidly by colonocytes, and are their major respiratory fuels (8). In rats, SCFA stimulate the growth of colorectal and ileal mucosal cells when they are delivered colorectally, intraperitoneally, or intravenously (9,10). It has been demonstrated that physiological concentrations of butyrate and acetate cause a significant increase in mucus secretion in the lumen of the isolated perfused rat colon (11). These observations lead to the hypothesis that fermentable complex carbohydrates, probably acting through SCFA, may promote the intestinal barrier function of preventing bacterial as well as endotoxin translocation under conditions such as stress and critical illness.

Resistant starch may have an additional advantage in that its fermentation appears to favor butyrate formation. It has shown that starch fermentation *in vitro* produces relatively more butyrate than the fermentation of non-starch polysaccharides (12). It has also suggested that nitrogen availability might be an important factor in determining SCFA production (13). There are two major sources of nitrogen for the large bowel microflora, urea (via the urease reaction) and proteins escaping from the small intestine. Of these, the latter can be altered most readily by diet and thus would seem to be a possible means for modifying fermentation products. It has been reported that large bowel fermentation of starch is altered by rice, potato, and soybean proteins (14). They support the hypothesis that non-digested protein, i.e., resistant protein, may control fermentation efficiency (14).

In the present study, therefore, an attempt was made to evaluate the physiological significance of feeding rats with potato peptide on the relationship between cecal SCFA productions. Moreover, we investigated the hepatoprotective effect of potato peptide in an experimental liver damage model induced by GalN in rats compared with the efficacy of soybean peptide.

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Materials and Methods

Preparation of potato peptides Potato peptide was

prepared by an enzymatic hydrolysis method. First, potato protein powder (450 kg) (Cosmo Foods Co., Ltd., Tokyo, Japan) was mixed with 4,500 L of water and 0.01 M NaOH. Then the mixture was stirred for 10 min at 50°C and allowed to stand at room temperature for 10 min, after which the supernatant was removed. Then 3,000 L of water was added to the precipitate, which was thereafter heat sterilized at 90-95°C for 15 min, and 2,000 L of water was again added to the mixture. The pH was adjusted to 10 by adding NaOH, and the mixture was hydrolyzed using an alkaline protease enzyme (Nagase Chemtex Corporation, Osaka, Japan) for 16 hr. Then the mixture was heated at 90-95°C for 15 min to denature the enzyme, and allowed to cool to terminate the enzyme reaction. Next, the mixture was filtered using diatomite, and the pH was adjusted to 5 with 3 L of acetic acid, and evaporated using a rotary evaporator (R-114; Sibata Scientific Technology Ltd., Tokyo, Japan). Following this, the mixture was ultra high temperature (UHT) sterilized and spray dried, and 113 kg of potato peptides was obtained. The average molecular weight of the mixture was 850 Da. Average molecular weight was determined as follows: first 10 mg of potato peptide was dissolved in 1 mL of Milli-Q, and then 0.5 μL of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich, Milwaukee, WI, USA) solution was added to a similar amount of peptide solution, after which the solution was air dried and analyzed by the matrix-assisted laser desorption ionization (MALDI) time of flight mass spectrophotometry (TOFMS) (Voyager-DE STR; Applied Biosystems, Foster, CA, USA). The details of the peptide preparation procedure are currently under patent review.

Animals and diets Male Fischer 344 rats (8 weeks old) were purchased from Charles River Japan (Yokohama, Japan). They were housed individually in cages. The animal facility was maintained on a 12-hr light-dark cycle at a temperature of 23±1°C and relative humidity of 60±5%. The rats were randomly assigned to 4 groups (n=5). There was no significant difference in the body weight among the groups at the beginning of the experiment. The composition of each diet is shown in Table 1. The compositions of soybean peptide and potato peptide powders were as follows (as %): moisture, 6.0 and 2.9; protein, 80.6 and 78.7; lipid, 3.2 and 0.6; carbohydrate, 4.7 and 12.5; ash, 5.5 and 5.3, respectively. Total moisture, protein, lipid, and carbohydrate contents were determined by the procedure of the AOAC (15). The amino acid compositions of casein, potato peptide, and soybean peptide are shown in Table 2. The amino acid compositions were determined as follows; first 4 mg each of casein, potato peptide, and soybean peptide samples was hydrolyzed in 2 mL of 6 M HCl at 110°C for 24 hr, vacuum dried, and then reconstituted with 1 mL of 0.2 M HCl, filtered (0.45-μm pore size, W-13-5; Tosoh, Tokyo, Japan) and analyzed with a Hitachi-8700 amino acid analyzer (Hitachi Ltd., Tokyo, Japan).

The experimental rats were fed for 2 week with a 20% casein diet (3,718 kcal/kg diet, control), in comparison with 2 diets containing either 20% potato peptide (3,657 kcal/kg diet) or 20% soybean peptide (3,652 kcal/kg diet). The soybean peptide was kindly provided by Fuji Oil Co., Ltd. (Osaka, Japan). After feeding with the experimental diets for 2 weeks, GalN was injected intraperitoneally at a dose of 400 mg/kg body weight in the control, soybean peptide, and potato peptide groups. GalN-untreated rats were injected with distilled water. At 22 hr after being injected with GalN, the rats were anesthetized by pentobarbital. Then the liver and blood were obtained. Blood samples were taken into tubes without an anticoagulant. After the samples stood at room temperature for 2 hr, sera were prepared by centrifugation at 1,200×g for 20 min. The liver was washed with cold saline, dehydrated on filter paper, and weighed before freezing for storage. The Animal Experimental Committee of Obihiro University of Agriculture and Veterinary Medicine approved this experimental design. All animal procedures described conformed to standard principles in Guide for Care and Use of Laboratory Animals (16).

Table 1. Composition of experimental diets (g/kg diet)

Ingredients	Dietary group[1]		
	CN	SP	PP
Casein	200	-	-
Soy peptides	-	200	-
Potato peptides	-	-	200
Soybean oil	70	70	70
Mineral mixture[2]	35	35	35
Vitamin mixture[3]	10	10	10
Cellulose powder	50	50	50
Sucrose	100	100	100
L-Cystine	3	3	3
Choline hydrogen tartrate	2.5	2.5	2.5
3-Butylhydroquinone	0.014	0.014	0.014
α-Corn starch	529.486	529.486	529.486
Caloric intake (kcal/kg diet)	3,718	3,652	3,657

[1]CN, Casein; SP, soy peptides; PP, potato peptides.
[2]AIN-93G mineral mixture.
[3]AIN-93G vitamin mixture.

Table 2. Amino acid compositions of experimental diets

Amino acids	Casein	Potato peptide	Soy peptide
	(mmol/g nitrogen)		
Asp	6.22	8.94	9.62
Thr	3.65	4.22	3.04
Ser	4.59	3.60	4.17
Glu	18.90	8.43	16.60
Gly	1.62	3.52	3.38
Cys	0.43	0.17	1.05
Val	5.94	4.12	3.65
Met	2.70	1.56	1.00
Ile	4.86	3.78	3.62
Leu	8.38	7.78	6.30
Tyr	5.00	3.38	3.07
Phe	4.59	3.96	4.15
Lys	7.16	4.93	4.96
His	2.70	1.27	2.10
Arg	3.24	3.54	6.47
Ala	2.70	3.96	3.37
Pro	10.10	3.54	4.42

Chemical analysis Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactic dehydrogenase (LDH) activities were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX, USA).

A part of the cecal content was taken out into desalting water in a vial without exposure to air, and suspended. The suspension of cecum was deproteinized with perchloric acid to form sodium salts of SCFA. Individual SCFA were measured by gas liquid chromatography (GLC) using 14A chromatograph (Shimadzu, Kyoto, Japan) with ZEBRON ZB FFAP capillary column (0.53 mm×30 m, Phenomenex, CA, USA) with H_3PO_4 (100 mL/L) as the liquid by the procedure of Hara *et al.* (17).

Fecal nitrogen content was determined by Kjeldahl's method. Apparent digestibility of protein was calculated as apparent protein digestibility=[(protein intake-fecal protein)/ protein intake]×100. Apparent digestibilities of soybean peptide and potato peptide were 93.5 and 93.9%, respectively, and thus lower than the 96% for casein.

Lipid peroxidation measurement Lipid peroxidation in the hepatic homogenate was assessed using the thiobarbituric acid-reactive substance (TBARS) method (18). The reaction mixture contained 0.2 mL of homogenate, 0.2 mL of 0.8% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid solution (pH 3.5) and 1.5 mL of 0.5% aqueous solution of thiobarbituric acid (TBA). The mixture was heated for 60 min in a boiling water bath. After cooling, 4 mL of 1-butanol was added and mixed vigorously. The organic phases were separated by centrifugation, and absorbance was measured by spectrophotometry (UV-1600; Shimadzu) at 532 nm. Protein content was determined as described by Lowry *et al.* (19).

Glutathione (GSH) concentration measurement The hepatic GSH concentration was determined by the method of Cohn and Lye (20). In brief, liver was homogenized in 10 mM ethylenediamine tetraacetic acid (EDTA) containing 5% trichloroacetic acid. The homogenate, consisting of 200 mg of liver in 2 mL of the buffer, was centrifuged at 1,500×g for 10 min to remove proteins, and then the supernatant was stored at −80°C until use. Four mL of 0.4 M Tris-HCl buffer (pH 8.9) containing 10 mM EDTA and 250 μL of *O*-phthalaldehyde in methanol (1 mg/mL, w/v) was added to 200 μL of the sample supernatant. After a 5 min reaction period, the GSH concentration was determined with a fluorometer (FP-6200; Jasco, Tokyo, Japan).

Growth of bacteria in the cecum Anaerobes, *Lactobacillus* and *Bifidobacterium* in the cecum were incubated for 5 days on GAM agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), Rogosa agar (Merck KGaA, Darmstadt, Germany) and BL agar (Eiken Chemical Co., Ltd., Tokyo, Japan) at 37°C by the gaspak method according to the procedure of Mitsuoka *et al.* (21-23).

Statistical analysis Data are presented as means and standard deviations. The significance of differences among treatment groups was determined by analysis of variance (ANOVA) with Duncan's multiple-range test (SAS Institute, Cary, NC, USA). Differences were considered significant at $p<0.05$.

Results and Discussion

Food intake, growth, liver weight, and cecal weight Table 3 shows body weight, food intake, and liver weight of rats fed potato peptide and soybean peptide. Body weight before administering GalN in the potato peptide group was significantly the lowest among the groups ($p<0.05$), and body weight after administering GalN in the potato peptide group was significantly lower than in the GalN-untreated group and GalN-treated with soybean peptide group ($p<0.05$). However, there was no significant difference in the negative body weight gain between the GalN-untreated group and the GalN-treated with potato peptide group, although there was a significant difference among all groups except the GalN-treated potato peptide group ($p<0.05$). Although food intake in the GalN-treated with potato peptide group was significantly lower than that of the GalN-untreated group ($p<0.05$), no difference was observed in food intake among the GalN-treated groups. There was no significant difference in liver weight among any groups. Cecal weight in the GalN-treated with soybean

Table 3. Body weight, food intake, liver and cecum weight in rats fed potato peptides and soybean peptides administering D-galactosamine

Components	Dietary group[1)]			
	CN	G-CN	G-PP	G-SP
Initial body weight (g)	175±6	174±5	173±6	175±5
BW before administering GalN (g)	229±8^{a}	231±9^{a}	218±6^{b}	232±6^{a}
Final body weight (g)	231±7^{a}	224±7^{ab}	216±10^{b}	228±10^{b}
Body weight gain (g)[2)]	1.9±1.3^{a}	-7.0±2.6^{b}	-2.1±4.3^{ab}	-4.3±5.9^{b}
Food intake (g/2 week)	250±37^{a}	229±8^{ab}	212±19^{b}	229±6^{ab}
Liver weight (wet g/100 g BW)	4.66±0.20	4.06±0.10	4.20±0.28	4.09±0.88
Cecum weight (wet g/100 g BW)	2.02±0.24^{a}	1.68±0.69^{ab}	2.23±0.30^{a}	1.34±0.45^{b}
Cecal pH	7.25±0.11^{bc}	7.69±0.16^{ab}	7.19±0.24^{c}	7.77±0.56^{a}

[1)]CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptides; G-SP, D-galactosamine-treated with soy peptides. Values are expressed as means±SD for 5 rats. ^{abc}Mean values within the same row bearing different superscripted roman letters are significantly different ($p<0.05$).
[2)]Body weight gain (g)=final body weight (g)−body weight before administering GalN (g).

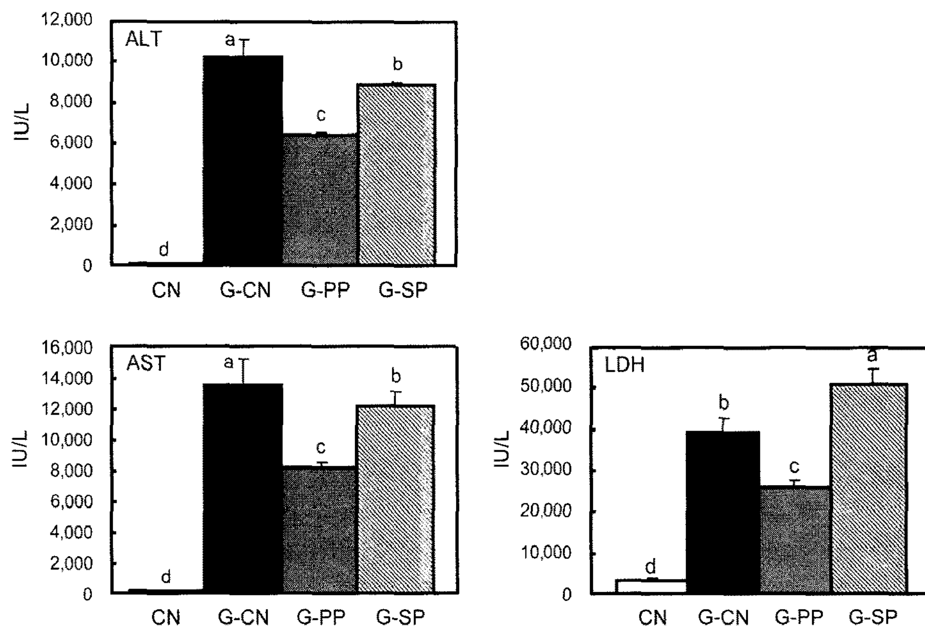

Fig. 1. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities in rats fed potato peptides and soy peptides after administration of GalN. Values are expressed as means for 5 rats, with standard deviations represented by vertical bars. [a-d]Mean values were significantly different, as determined by Duncan's multiple-range test ($p<0.05$). CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptide; G-SP, D-galactosamine-treated with soy peptide.

peptide group was significantly lower than that in the GalN-untreated group and GalN-treated with potato peptide group ($p<0.05$) , and cecal pH in the GalN-treated with potato peptide group was significantly lower that in the other GalN-treated groups ($p<0.05$).

Liver damage indices Figure 1 shows serum ALT, AST, and LDH activities of rats fed potato peptide and soybean peptide after GalN administration. It is well known that administration of GalN causes an increase in the level of serum transaminases, hepatic necrosis, and coma (4,5). In the present study, serum ALT, AST, and LDH levels in the 3 GalN-treated groups were significantly higher than those in the GalN-untreated group ($p<0.05$). As shown in Fig. 1, serum ALT, AST, and LDH activities were increased approximately 282-, 96-, and 13-fold, respectively, in the GalN-treated control group compared with the GalN-untreated group, respectively. Serum ALT and AST levels in the GalN-treated with potato peptide and soybean peptide groups were significantly lower by approximately 37 and 13, and 40 and 10%, respectively, than in the GalN-treated control group ($p<0.05$). Serum LDH activity that was dramatically increased by GalN treatment was significantly decreased approximately 34% by the potato peptides ($p<0.05$). However, serum LDH activity in the GalN-treated with soybean peptide group was significantly higher than in the GalN-treated control group ($p<0.05$). One of the major findings of this study was that consumption of a diet containing 20% potato peptide consistently reduced GalN-induced liver damage in rats. This was manifest as blunting of the increases in serum transaminase activities. It has been reported that GalN induces liver damage by inhibiting the synthesis of RNA and protein through a decrease in the cellular UTP concentration (1, 2). Stachlewitz *et al.* (24) also concluded that early stage endotoxin influx and Kupffer cell activation exacerbated the hepatotoxic action of GalN itself at a later stage. This conclusion was based on the observation that monoclonal antibodies to endotoxin or antisera to tumor necrosis factor (TNF)-α effectively blocked the increases in serum transaminase activities at 24 hr after GalN administration. Morita *et al.* (25) have reported that high-amylose cornstarch (HAS) ingestion significantly reduces the increase in serum transaminase activities at 22 hr after the injection of GalN. They also reported that the portal endotoxin concentrations were significantly and positively correlated with the serum concentration of TNF-α and serum ALT activity (25). Although there were no portal endotoxin data and serum TNF-α data in this study, this serum transaminase for rats fed cornstarch with potato peptides were similar to the data in rats fed the HAS diet (25). The cecal increasing SCFA for rats fed the HAS diet (25) significantly reduces the liver damage and has greater cecal SCFA production as measured by pool size than those fed the basal diet in rats treated with GalN. In this study, soy peptide was not increased the cecal SCFA concentration. Therefore, potato peptide, which increased the cecal SCFA might have hepatoprotective activity against liver damage induced by GalN in rats.

Cecal bacteria and short-chain fatty acid levels Table 4 shows cecal anaerobe, *Bifidobacterium*, and *Lactobacillus* levels in rats fed potato peptide and soybean peptide after administration of GalN. Anaerobe levels in the 3 GalN-treated groups were significantly higher than that in the GalN-untreated group ($p<0.05$). *Bifidobactrium* level in the GalN-treated with potato peptide was significantly higher than in the GalN-untreated group and GalN-treated with soybean peptide group ($p<0.05$). *Lactobacillus* level in the

Table 4. Cecal bacterial populations in rats fed potato peptides and soybean peptides administering D-galactosamine

Components	Dietary group[1)]			
	CN	G-CN	G-PP	G-SP
	Log$_{10}$ CFU of bacteria/wet g cecum			
Anaerobe	6.88±0.52^{b}	7.40±0.14^{a}	7.67±0.11^{a}	7.38±0.18^{a}
Bifidobacterium	6.37±0.28^{bc}	6.62±0.14^{ab}	6.93±0.09^{a}	6.00±0.48^{c}
Lactobacillus	2.25±0.39^{d}	2.90±0.48^{c}	4.75±0.19^{b}	5.33±0.37^{a}

[1)]CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptides; G-SP, D-galactosamine-treated with soy peptides. Values are expressed as means±SD for 5 rats. [a-d]Mean values within the same row bearing different superscripted letters are significantly different ($p<0.05$).

Table 5. Cecal short-chain fatty acid concentration in rats fed potato peptides and soybean peptides administering D-galactosamine

Components	Dietary group[1)]			
	CN	G-CN	G-PP	G-SP
	mmol/wet g			
Acetic acid	14.85±2.42^{a}	10.23±3.60^{b}	14.86±3.60^{a}	10.24±1.78^{b}
Propionic acid	4.21±1.37^{b}	4.14±1.37^{b}	6.96±1.86^{a}	4.00±0.63^{b}
n-Butyric acid	2.09±0.99^{b}	1.42±0.47^{b}	3.60±0.80^{a}	1.51±0.47^{b}
Total SCFA[2)]	21.15±4.32^{ab}	15.81±5.10^{b}	25.42±5.19^{a}	15.75±2.63^{b}

[1)]CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptides; G-SP, D-galactosamine-treated with soy peptides. Values are expressed as means±SD for 5 rats. [a-c]Mean values within the same row bearing different superscripted letters are significantly different ($p<0.05$).
[2)]Total SCFA =acetic acid+propionic acid+*n*-butyric acid.

GalN-treated with potato peptide and soybean peptide groups were significantly higher than that in the GalN-untreated group and GalN-treated control group ($p<0.05$), and that in the GalN-treated with soybean peptide group was the highest among the groups. Morita *et al.* (14) reported that rats fed a low-amylose cornstarch (LAS) diet with potato protein and HAS diet with potato protein had significantly greater cecal butyrate level than those fed a casein-LAS diet and casein-HAS diet, and large bowel fermentation of starch was altered by dietary protein. Furthermore, these data support the hypothesis that potato protein may control fermentation efficiency as well as the fermentation profile of HAS, possibly as a result of a change in microflora through the change in the ratio of starch to nitrogen in the cecum. In this study, *Bifidobactrium* level in the GalN-treated with potato peptide group was significantly higher than in the GalN-treated control group by Student's *t*-test ($p=0.003$), and *Lactobacillus* level in the GalN-treated with potato peptide and soybean peptide groups were significantly higher than in the GalN-untreated and -treated control groups ($p<0.05$). Therefore, it may be possible that potato peptide positively affected the cecal microflora composition in the study.

Table 5 shows cecal SCFA concentrations in rats fed potato peptide and soybean peptide after administering GalN. Acetic acid, propionic acid, butyric acid, and total SCFA concentrations in the GalN-treated with potato peptide group were significantly higher than those in the GalN-treated control group and GalN-treated with soybean peptide group ($p<0.05$). Barcelo *et al.* (11) reported that SCFA stimulates mucin secretion by the isolated rat colon. In the present study, cecal SCFA, *Bifidobacterium*, and *Lactobacillus* levels were greater in rats fed the 20% potato peptide diet. On this basis, there was considerably more SCFA available to supply the cecum in rats fed this diet and to promote cecal and colonic function. Kasravi *et al.* (26) reported that oral supplementation of lactobacilli showed the moderate effect in the prevention of GalN-induced liver damage and bacterial translocation. Additionally, it has been shown that a rice-based diet, possibly including resistant starch, assists in reducing intestinal colonization of *Brachyspira pilosicoli* in piglets, thus protecting them from clinical sequelae (27). In this study, the cecal SCFA data for rats fed potato peptide were similar to the data in rats fed the HAS diet (25), which has been shown that HAS ingestion significantly reduces the liver damage and has greater cecal SCFA production as measured by pool size than those fed the basal diet in rats treated with GalN. Therefore, in this study the increased cecal fermentation by potato peptide might be related with the reduction of liver damage in rats treated with GalN.

Hepatic glutathione and lipid peroxide Figure 2 shows hepatic GSH concentration in rats fed potato peptides and soybean peptides after administering GalN. Hepatic GSH concentration in the 3 GalN-treated groups was significantly lower than that in the GalN-untreated group ($p<0.05$). However, hepatic GSH concentration in the potato peptide group was significantly increased than that in the GalN-treated control group ($p<0.05$). Recently, it has been suggested that reactive oxygen species (ROS: OH·, O_2^-, RO·, ROO·, $^1O_2$) produced by activated hepatic macrophages might be the primary cause in GalN-induced liver damage (28, 29). Quintero *et al.* (3) also reported that intracellular free radical production gradually increases with the GalN concentration in rat hepatocytes. In this study, the significant difference of hepatic TBARS level in GalN-treated control group was not found with that in GalN-untreated group, but the level in the GalN-treated control group was slightly increased than that in the GalN-

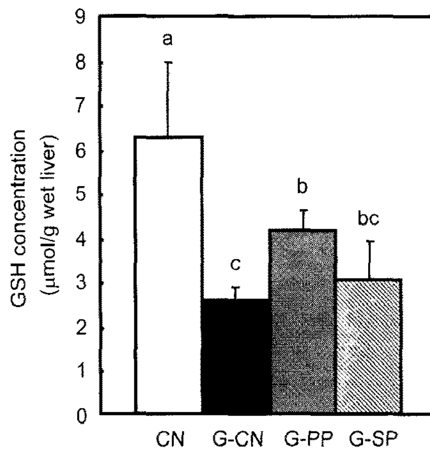

Fig. 2. Hepatic glutathione concentration in rats fed potato peptides and soy peptides after administering GalN. Values are expressed as means for 5 rats, with standard deviations represented by vertical bars. [abc]Mean values were significantly different, as determined by Duncan's multiple-range test (p<0.05). CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptide; G-SP, D-galactosamine-treated with soy peptide.

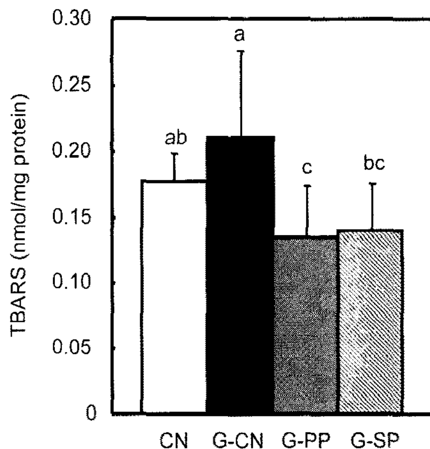

Fig. 3. Hepatic TBARS concentration in rats fed potato peptides and soy peptides after administering GalN. Values are expressed as means for 5 rats, with standard deviations represented by vertical bars. [abc]Mean values were significantly different, as determined by Duncan's multiple-range test (p<0.05). CN, Casein; G-CN, D-galactosamine-treated with casein; G-PP, D-galactosamine-treated with potato peptide; G-SP, D-galactosamine-treated with soy peptide.

untreated group (Fig. 3). Furthermore, hepatic TBARS level in the GalN-treated with potato peptide and soybean peptide groups was significantly decreased than that in the GalN-treated control group (p<0.05). It is interesting that the TBARS level in the GalN-treated with potato peptide group was also significantly lower than that in the GalN-untreated group (p<0.05). But, the precise reason by which potato peptide more decreased hepatic TBARS level than GalN-untreated group is not entirely clear. However, it is certain that the potato peptide effectively attenuated the increased hepatic TBARS value in rats treated with GalN. Therefore, it is considerable that the reduction of liver damage induced by GalN might be related with the increased hepatic GSH level and cecal SCFA productions by increasing the intestinal lactobacilli (26). However, we could not clarify the mechanism in this study.

In conclusion, the results of this study demonstrate that potato peptide has hepatoprotective effects against GalN-induced liver damage in rats via promotion of cecal SCFA, *Bifidobacterium*, *Lactobacillus* levels, and hepatic GSH level, and reduction of the hepatic TBARS level. Therefore, the potato peptide contributes greatly to the dietary health of humans by providing substantial amounts of antioxidants, though further work is necessary to clarify the hepatoprotective mechanisms of potato peptides against GalN-induced liver injury.

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