Note

Antioxidative Activity and Protective Effect against Ethanol-Induced Gastric Mucosal Damage of a Potato Protein Hydrolysate

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Summary Antioxidative activity and protective effect against ethanol-induced gastric mucosal damage of potato protein hydrolysate (potato peptides, Po-P) were studied in vitro and in vivo. The Po-P obtained by proteolysis with Amano P and pancreatin inhibited linoleic acid oxidation either by 83% at its coexistent 0.005% in a ferric thiocyanate assay system or by 32% at its coexistent 0.0002% in a \( \beta \)-carotene decolorization assay system. Meanwhile Po-P were orally administered to male Wistar rats at doses of 12.5–100 mg/kg of body weight (BW) 30 min prior to ethanol injection. Consequently the ethanol-induced gastric damage was significantly reduced in a dose-dependent manner in the Po-P administered rat. The highest effect was observed in the group dosed with 100 mg Po-P/kg BW; the inhibition ratio was 69.6%. The extent of antioxidation or protection against ethanol-induced gastritis was quite similar to those of the respective peptides from casein, corn protein and ovalbumin, suggesting that the potato protein hydrolysate could serve as a useful food ingredient in practical eating habits.

Key Words antioxidative activity, potato protein hydrolysate, ethanol-induced gastric mucosal damage

Free radicals and reactive oxygen species are known to play crucial roles in protection against microbial infection. However, they often injure host cells or tissues and trigger off several diseases such as carcinogenesis (1, 2), Alzheimer disease (3, 4), Parkinson disease (4) and cardiovascular disease (5).

Especially in food products containing more or less lipids, oxidation is a critical problem in view of food deterioration. So far various synthetic and natural antioxidants have been used to prolong the shelf life of foods. Food components having protective effects against oxidative stress in vivo are particularly interesting among them.

In this connection, casein (6), soy protein (7), ovalbumin (8) and their peptide derivatives (9–11) have antioxidative activity. We have been occupied in developing techniques for the application of biologically active substances from agricultural byproducts (12–14). For example, the residue deprived of starch from potato includes much protein and its utilization is highly expected.

We found that the hydrolysate from potato protein had preliminarily an antioxidative activity. In the present paper, we evaluated its antioxidative activity in vitro and its protective effect against ethanol-induced gastritis in vivo.

Materials and Methods

Preparation of potato protein hydrolysate. A potato extract solution was heated at 60°C for 30 min and centrifuged to obtain a protein precipitate. The precipitate was used to prolong the shelf life of foods. Food components having protective effects against oxidative stress in vivo are particularly interesting among them.

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4 mL of ethanol containing 1.25% linoleate and 0.05% Tween 80 and 2 mL of 0.025, 0.05 or 0.1% test peptide solution was incubated at 50°C for 168 h, of which an aliquot (0.1 mL) was taken out into a test tube every 24 h. Then, 9.7 mL of 75% ethanol solution, 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 0.02 M iron (II) chloride solution were added to each tube, followed by measurement of the optical density at 500 nm. In the above assay system, 2 mL of BHA solution was added as a reference standard instead of a test peptide solution. The inhibition ratio (IR) was calculated from the following formula.

\[
\text{IR (\%) = 100 - \left( \frac{O.D.500 \text{ of sample}}{O.D.500 \text{ of control}} \right) \times 100}
\]

In the β-carotene bleaching assay system, 3.4 mL of a chloroform solution containing linoleate (100 mg/mL), β-carotene (1 mg/mL) and Tween 40 (200 mg/mL in the 1:2.5:5 ratio) was evaporated to dryness in vacuo, followed by blending with 0.2 M phosphate buffer of pH 6.8 (17.8 mL) and distilled water (200 mL). Subsequently, 4.9 mL of the blending solution was added to 0.1 mL of test or reference solution containing 0.0025, 0.005 or 0.01% peptide or BHA. The optical density at 470 nm was monitored every 10 min between 0 and 40 min at 50°C. The inhibitory ratio (IR) was calculated from the following formula (18):

\[
\text{IR (\%) = \left( \frac{100 - Pc}{100 - Ps} \right) \times 100}
\]

\[
\text{Pc} = \left( \frac{O.D.470 \text{ of control at 40 min}}{O.D.470 \text{ of control at 0 min}} \right) \times 100
\]

\[
\text{Ps} = \left( \frac{O.D.470 \text{ of sample at 40 min}}{O.D.470 \text{ of sample at 0 min}} \right) \times 100
\]

**Measurement of the protective effect against ethanol-induced gastritis.** The antioxidative activity in vivo was evaluated using a rat model of ethanol-induced acute ulcer (19, 20). Male Wistar rats of 6 wk age were fed the usual 25% casein diet (mineral and vitamin premix, AIN-76 likeness (21, 22)) for 3 d and deprived of food for a full day. Then, each test sample dissolved in 5% arabic gum solution was orally administered in doses of 12.5–100 mg/kg BW. Thirty minutes later, the rats were provided with absolute ethanol corresponding to 5 mL/kg BW, from which the stomachs were excised under anesthesia with intraperitoneal injection of 5% nembutal (1 mL/kg BW) in 1 h. Each stomach excised was distended and immersed in 5% formalin after removal of the contents and then was opened along the line of greater curvature and spread on filter paper. The degree of gastritis was observed by naked eye, followed by image-analysis using Adobe Photoshop LE-J as well as NIH Image and representation in % of lesion area in mucosa of stomach. The inhibition ratio (IR) was calculated as follows:

\[
\text{IR (\%) = \left[ \left( \frac{\text{Damage ratio of control}}{\text{Damage ratio of test sample group}} \right) / \left( \frac{\text{Damage ratio of control}}{\text{Damage ratio of control}} \right) \times 100 \right]}
\]

This experimental design was approved by the Animal Experiment Committee of Rakuno Gakuen University and the rats were managed in line with the Guide for the Care and Use of Laboratory Animals.

**Statistical analysis.** Data on animal experiment were expressed as the means±SE (n=6) and analyzed by two-way ANOVA with the aid of Stat View 5 software for Macintosh, among which the differences were considered significant at p<0.05.

**Results and Discussion**

The inhibition ratios against linoleate peroxidation of Po-P, Cr-P, Ov-P, Ca-P and Cl-P at a concentration of 0.005% common to all samples were obtained as 88, 82, 75, 80 and 50%, respectively, by the ferric thiocyanate method (Fig. 1). The antioxidative activity of Po-P was similar to those of Cr-P and Ca-P, but higher than those of Ov-P and Cl-P.

**Fig. 1.** Antioxidative activity of potato and other peptides in a linoleic acid auto-oxidation system measured by ferric thiocyanate method. The reaction mixture containing linoleic acid and the peptide was incubated 50°C for 168 h. Open, gray and solid bars represent 0.0025, 0.005 and 0.01% of the peptide concentration or BHA, respectively.

**Fig. 2.** Inhibitory ratio of potato and other peptides on a linoleic acid oxidation system measured by β-carotene bleaching method. The reaction mixture containing linoleic acid-β-carotene and the peptide was incubated 50°C for 40 min. Open, gray and solid bars represent 0.0005, 0.0001 and 0.0002% of the peptide concentration, respectively.
Fig. 3. Effect of potato and other peptides on gastric mucosal damage induced by ethanol in rats. Each peptide was administered orally to the rats at the dose of 100 mg/kg BW 30 min prior to ethanol injection. The height and bar of each column represents the means±SE (n=6); those not sharing a common superscript letter are significantly different at p<0.05.

Fig. 4. Effect of dose amount of potato peptide on gastric mucosal damage induced by ethanol in rats. The potato peptide was administered orally to the rats 30 min prior to ethanol injection. The height and bar of each column represents the means±SE (n=6); those not sharing a common superscript letter are significantly different at p<0.05.

Fig. 5. Representative images of gastric mucosal damage induced by ethanol in rats. The potato peptide was administered orally to the rats at the dose of 50 mg/kg BW 30 min prior to ethanol injection.

In the β-carotene bleaching method, the inhibitory ratio of Po-P, Cr-P, Ov-P, Ca-P and Cl-P at a concentration of 0.0002% was 32, 25, 22, 34 and 11%, respectively (Fig. 2). There were some differences among these peptide samples about the antioxidative activity in the two types of assay systems. Po-P and Ca-P showed a relatively high antioxidative activity in both assay systems. Cr-P exerted more antioxidative effect in the ferric thiocyanate method than in the β-carotene bleaching method, while Ov-P and Cl-P exhibited lower activities than others in both of the methods. Although the difference in inhibitory activity between both assay systems seems to be connected with amino acid sequence and/or composition of the samples including active peptides, the details remain obscure.

The area ratio of ethanol-induced gastric mucosal damage in the group given 100 mg Po-P/kg BW was 4.8%, which was significantly lower than 15.8% in the control group (Fig. 3). The calculated inhibition ratios of Po-P, Cr-P, Ov-P, Ca-P and Cl-P at the same dose were 69.6, 65.8, 57.6, 72.2 and 22.7%, respectively. The efficacy of Po-P was similar to those of Cr-P and Ca-P, being higher than those of other peptide samples.

Next, we examined the dose-dependency of Po-P against gastritis. Administration of Po-P significantly inhibited the ethanol-induced gastric mucosal damage in a dose-dependent manner in the range from 12.5 to 100 mg/kg BW (Fig. 4). The inhibition ratios at doses of...
12.5, 25, 50 and 100 mg/kg BW were 41.7, 44.8, 53.3 and 69.6%, respectively. Typical images of ethanol-induced gastritis in both control and Po-P groups are shown in Fig. 5. A severe necrotic injury was observed on the mucosa of the stomach in the control rat. In this contrast, Po-P administration markedly reduced the injury.

A rat model of ethanol-induced gastric mucosal damage is widely used for developing and evaluating antioxidative compounds. It has been shown that lipid peroxidation is significantly increased by ethanol infusion into the stomach and closely related to the incidence of gastric damage (23, 24). Furthermore, antioxidants capable of inhibiting lipid-peroxide formation are well-known to exert a protective effect in this model (25). There are, however, different results on participation of reactive oxygen species in damage development. Szelényi and Brune (19) and Terano et al. (20) have reported that free radicals derived from oxygen are involved in pathogenesis in this model, while Mizui et al. (25) showed that a ferrous ion and xanthine oxidase system, besides oxygen radicals, contributed to lipid peroxidation and lesion formation. In any case, the protective effect of Po-P against ethanol-induced gastric mucosal damage may be due to its antioxidative activity inhibiting lipid-peroxide formation.

In the present study, we found that Po-P exerted antioxidative activity in vitro and a protective effect against ethanol-induced gastric mucosal damage in vivo. Autoxidation of polyunsaturated fatty acids causes the formation of hydroperoxides and aldehydes not only in foods but also in the biological system (26). Our results suggested that Po-P could serve as a potent antioxidative ingredient for the prolongation of lipid food shelf life or a protectant against cell and tissue damages by oxidative stress in vivo. Further work is planned to identify active peptides from the hydrolysate.

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