Dietary Supplementation with Pearl Barley (Adlay, Coix lacryma-jobi L. var Ma-yuen Stapf) Extract Increases Oxidation Resistance in the Liver of Rats \textit{ex vivo}

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Received May 14, 2010; Accepted August 3, 2010

The antioxidative fraction of roasted pearl barley (adlay, Coix lacryma-jobi L. var Ma-yuen Stapf), which is used in commercial tea beverages, was obtained by column chromatography and eluted with 70% ethanol. We investigated the effect of dietary supplementation with pearl barley extract on the formation of thiobarbituric acid-reactive substances in rat serum and \textit{ex vivo} tissues. We also investigated whether the serum and tissue homogenates were susceptible to lipid peroxidation. Rats that were fed a diet containing 5 g/kg pearl barley extract (antioxidative fraction) for 14 days exhibited no significant reduction in the formation of thiobarbituric acid-reactive substances in the serum. However, compared to controls, liver homogenates from rats fed pearl barley extract showed significantly lower susceptibility to lipid peroxidation (induced by 2,2′-azobis(2-amidinopropane)hydrochloride). These results demonstrate that a diet containing pearl barley extract increases oxidation resistance in \textit{ex vivo} rat livers.

Keywords: pearl barley, adlay, oxidation resistance, \textit{p}-coumaric acid

Introduction

Reactive oxygen species such as hydroxyl radicals, superoxide anion radicals, and singlet oxygen are believed to be involved in carcinogenesis, mutagenesis, aging, and atherosclerosis (Cutler, 1984; Pryor, 1986; Harman, 1992; Stadtman, 1992; Ames \textit{et al.}, 1993; Packer, 1995). Endogenous antioxidants are presumed to protect cells against reactive oxygen species (Cutler, 1984; Ames \textit{et al.}, 1993; Packer, 1995), but there is increasing interest in the protective function of natural antioxidants found in dietary plants (Osawa \textit{et al.}, 1995), particularly in those that are known to have medicinal benefits.

Since ancient times, pearl barley (adlay, Coix lacryma-jobi L. var Ma-yuen Stapf), a true grass of Chinese and Indo-Chinese origin, has been used as a tonic in the East. Recently, it has been used in commercial tea beverages. Research on the medicinal properties of pearl barley has shown that this grass has great potential as a high value-added product thereby attracting international attention. The compound coixenolide has been shown to have growth-inhibiting activity against Ehrlich ascites tumor cells in mice (Tanumura, 1961; Ukiah and Tanumura, 1961).

To the best of our knowledge, no studies have investigated the benefits of supplementation of animal diet with pearl barley extract and whether this improves their \textit{in vivo} antioxidant status. In the present study, we evaluated the effect of a pearl barley antioxidative extract on lipid peroxidation and oxidation resistance in \textit{ex vivo} tissues of rats.

Materials and Methods

\textbf{Roasted pearl barley} Pearl barley (adlay, Coix lacryma-jobi L. var Ma-yuen Stapf) was roasted and ground at Chanson Cosmetics Co. Ltd (Shizuoka, Japan).

\textbf{Extraction of pearl barley antioxidative fraction} Roasted pearl barley was stirred in hot water (30 times the volume of the barley) at 90°C for 12 min. The extract was strained through a stainless steel wire mesh screen (opening, 180 μm) and filtered through a filter paper (Advantec No. 2; Toyo Roshi Kaisha Ltd.). The filtrate was charged on a Diaion HP2MG column (300 g, 35 φ × 310 mm; Mitsubishi Kasei Co., Ltd.) and
eluted with distilled water with a stepwise increase in ethanol concentration. Each fraction contained 5 bed volumes. The extraction procedure is outlined in Fig. 1. Fraction 3 was concentrated under vacuum and freeze-dried. This extract was evaluated as the antioxidative fraction.

**Animals and diet** The protocols for animal experiments were approved by the Laboratory Animal Care Advisory Committee of Nagoya University. Five-week-old male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) housed individually in stainless steel wire mesh cages were used in the experiments. The rats were allowed free access to water and a semi-purified diet. Rats in the control group were fed a normal diet of AIN-93G, and those in the experimental group were fed a diet containing 5 g/kg pearl barley extract.

**Experimental design and tissue preparation** All animals were fed the control diet (AIN-93G) for 7 days before the experiment. We divided 12 rats into 2 groups and fed them the prescribed diet for 14 days. Thereafter, the rats were sacrificed by decapitation. The collected blood was kept at room temperature for 5 min (to allow it to coagulate), and the serum was obtained by centrifugation at 1,600 × g for 15 min at 4°C. Serum separation was accomplished within 30 min, and the serum was immediately stored at −80°C. Tissues were rapidly excised after being perfused with physiological saline solution. The tissues were kept at −80°C until the assay. These conditions were standardized for all animals.

**Erythrocyte ghost system** Erythrocyte ghosts were prepared from rabbit blood cells according to the method of Osawa et al. (1987). The induction of lipid peroxidation was carried out with tert-butyl-hydroxy peroxide (t-BHP), according to the method of Ames et al. (1981). Test samples were dissolved in methanol, and t-BHP was added. After incubation, the amount of thiobarbituric acid-reactive substances (TBARS) was determined based on absorbance at 532 nm for malondialdehyde (MDA) (Osawa et al., 1987).

**Prevention of peroxynitrite-derived collagen modification** The in vitro effect of test samples on peroxynitrite-derived collagen modification was examined using a polyclonal antibody specific to 3-nitrotyrosine, according to the method of Kato et al. (1997). The test samples were dissolved in methanol, and the antibody was added.

**Quantitative analysis of antioxidative activity** The IC₅₀ (half-maximal (50%) inhibitory concentration) was calculated by a logistic curve according to equations 1 and 2.

\[
Z = \ln \left( \frac{IR}{1-IR} \right) \quad \text{(Logistic conversion)} \quad \text{Eq. 1}
\]

\[
Z = F \left( \ln \left( \text{Conc} \right) \right) \quad \text{Eq. 2}
\]

IR: Inhibition rate of peroxidation
Conc: Concentration of evaluation sample

**Measurement of oxidation resistance in serum** Serum samples were oxidized at 37°C by incubation with 2,2′-azobis(2-amidinopropane)hydrochloride (AAPH) (final concentration, 25 mM). During incubation, aliquots were taken at specific time intervals, and lipid peroxidation was inhibited by the addition of butylated hydroxytoluene (BHT) (final concentration, 60 μM). The degree of oxidation was immediately measured by the TBARS assay (Naito and Yamakawa, 1978). All results are expressed as the mean (standard error (SE)). Data were compared using Student’s t test. Probability values less than 0.05 were considered to be significant.

**Measurement of oxidation resistance in tissues** Frozen tissue (0.5 g) was homogenized in 20 volumes of 20 mM phosphate buffer (pH 7.4) using a Potter-Elvehjem homogenizer. The homogenate was oxidized at 37°C by incubation with AAPH (final concentration, 25 mM). During incubation, aliquots were taken at specific time intervals, and lipid peroxidation was inhibited by the addition of BHT (final concentration, 60 μM). The degree of oxidation was immediately measured by the TBARS assay (Ohkawa et al., 1979). All results are expressed as the mean (standard error (SE)). Data were compared using Student’s t test. Probability values less than 0.05 were considered to be significant.

**High-performance liquid column chromatography and structure elucidation** High-performance analysis was carried out using an octadecyl silica (ODS) column (Develosil ODS-HG-5, 20 μm × 250 mm; Nomura Chemical Co., Ltd.). The samples were eluted in an acetonitrile gradient (15–35%) in 0.1% v/v trifluoroacetic acid. Ultra-violet detection was performed by absorbance at 280 nm. 1H-nuclear magnetic
resonance (NMR) spectra were obtained using a Bruker ARX 400 spectrometer. Mass spectrometry (MS) was performed on a Joel JMS 700 MStation spectrometer.

Results

Extraction of the antioxidative fraction from pearl barley
Roasted pearl barley was extracted with hot water at a rate of 12.5%. The antioxidative fraction (Fraction 3) was eluted from the Diaion HP2MG column with 70% ethanol as shown in Table 1.

Effect of dietary supplementation with the antioxidative fraction on body weight gain, food intake, and liver weight
There was no significant difference in body weight gain or food intake between the experimental and control groups. Liver, kidney, gastrocnemius muscle, and brain weight also did not differ between the groups after 14 days (data not shown). These results suggest that dietary supplementation with the pearl barley antioxidative fraction did not cause serious toxicity in rats.

Effect of dietary supplementation with the antioxidative fraction on TBARS concentration in serum and tissue homogenates
Serum TBARS concentration was slightly lower in the experimental group (80% that of the control group); however, the difference was not significant. The TBARS concentration of tissue homogenates in the experimental group was lower than that of tissue homogenates in the control group (80% and 90% that of the liver and kidney homogenates in the control group).

Effect of dietary supplementation with the antioxidative fraction on susceptibility to lipid peroxidation
Figure 2 shows the concentration of TBARS in serum and liver homogenates exposed to AAPH. There was a significant difference in the liver TBARS concentration between the groups (p < 0.05). After 4 h of incubation, lipid peroxidation in livers from the experimental group was inhibited by 75% compared to the control group. No significant differences were observed between the groups with respect to TBARS concentration in the serum and kidney (data not shown). The susceptibility of serum and kidney to AAPH lipid peroxidation was not affected by dietary pearl barley extract.

Major peak of antioxidative fraction
The major peak in the antioxidative fraction was identified as p-coumaric acid according to MS and 1H-NMR data, constituting 18% of the fraction.

<table>
<thead>
<tr>
<th>Yield [g]</th>
<th>Antioxidative activity on RBC ghost oxidation induced by t-BHP IC50 [mg/mL]</th>
<th>Inhibition activity on nitration of collagen by peroxynitrate IC50 [mg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water extract</td>
<td>33.46</td>
<td>1.0</td>
</tr>
<tr>
<td>Fr.1 H2O elute</td>
<td>15.81</td>
<td>0.82</td>
</tr>
<tr>
<td>Fr.2 30% EtOHelute</td>
<td>7.09</td>
<td>0.82</td>
</tr>
<tr>
<td>Fr.3 70% EtOH elute</td>
<td>4.08</td>
<td>&lt; 0.13</td>
</tr>
<tr>
<td>Fr.4 EtOH elute</td>
<td>0.03</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of dietary supplementation with pearl barley extract on the susceptibility of serum and liver homogenates to lipid peroxidation induced by 2,2′-(2-amidinopropane)hydrochloride (AAPH) in rats. Values are the mean (SE).
* Significantly different (p < 0.05) from control values.
Discussion

The antioxidative activity of phenolic phytochemicals has been widely investigated, and our laboratory has focused on natural antioxidants. In the present study, an antioxidative fraction of roasted pearl barley was obtained by column chromatography using 70% ethanol extraction. This fraction showed antioxidant activity in a hydrophilic system using erythrocyte ghosts; inhibition of nitration was also observed. The inhibition of peroxynitrite-derived nitration of protein and lipid peroxidation may aid the prevention of biological disorders or diseases. Therefore, we sought to determine whether the pearl barley antioxidative fraction act as an in vivo antioxidant when fed to rats.

The serum TBARS concentration was slightly lower in rats fed the barley extract than in rats fed the control diet; the difference was not significant. Nevertheless, the susceptibility of liver homogenates to AAPH-induced lipid peroxidation was significantly affected on supplementing the rat diet with the pearl barley antioxidative extract. We isolated p-coumaric acid, a typical phenolic antioxidant from this fraction. Competitive reaction has been suggested as one of the inhibition mechanisms by phenolic antioxidants (Kato et al. 1997). We conclude that the antioxidant activity of pearl barley includes phenolic antioxidants.

In summary, our results suggest that dietary supplementation with pearl barley extract increases oxidation resistance in the ex vivo rat liver. Further studies are needed to understand the mechanism by which dietary supplementation with antioxidants, such as those from pearl barley, confers oxidation resistance to the liver.

Acknowledgments The authors are grateful to Drs. Michitaka Naito and Yoshiko Fukuchi from the School of Life Studies, Sugiyama Jogakuen University, Nagoya, Japan, for their assistance in the animal experiments.

References


