Preventive Effects of Dietary Cabbage Acylated Anthocyanins on Paraquat-induced Oxidative Stress in Rats

Kiharu IGARASHI,† Yuriko KIMURA, and Asako TAKENAKA

Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka-shi, Yamagata 997-0037, Japan

Received January 11, 2000; Accepted March 30, 2000

The preventive effects of acylated anthocyanins from red cabbage on paraquat-induced oxidative stress were determined in rats. Decreased food intake and body weight gain, and increased lung weight and atherosclerotic index by feeding the rats on a diet containing paraquat were clearly suppressed by supplementing acylated anthocyanins to the paraquat diet. Paraquat feeding increased the concentration of thiobarbituric acid-reactive substances (TBARS) in liver lipids, and decreased the liver triacylglycerol level. These effects tended to be suppressed by supplementing acylated anthocyanins to the paraquat diet. In addition, the catalase activity in the liver mitochondrial fraction was markedly decreased by feeding on the paraquat diet, this decrease being partially suppressed by supplementing the paraquat diet with acylated anthocyanins. An increase in the NADPH-cytochrome-P450-reductase activity in the liver microsome fraction by paraquat was suppressed by supplementing the paraquat diet with acylated anthocyanins. These results suggest that acylated anthocyanins from red cabbage acted preventively against the oxidative stress in vivo that may have been due to active oxygen species formed through the action of paraquat.

Key words: red cabbage; acylated anthocyanins; oxidative stress; paraquat

The physiological functions of polyphenols are receiving a lot of positive attention because the French paradox is closely related to the large amount of polyphenols in red wine.11 Anthocyanins are contained in many fruits, vegetables and cereals with red skins, as polyphenols, and are also contained in red wine. Accordingly, the antioxidative and physiological functions of anthocyanins have been focused on by many researchers. However, the preventive effects of anthocyanins against oxidative stress in vivo have not been fully examined. We have previously found malvin, rubrobrassinin, and nasunin to be the major anthocyanins in wild grapes,20 atsumi-kabu (a variety of red turnip),21 and eggplant,22 respectively. These anthocyanins had antioxidative activity toward the autoxidation of linoleic acid and the oxidation of linoleic acid with lipoygenase.23,24 Therefore, it is of great interest to determine the in vivo antioxidative activity and physiological functions of anthocyanins which may be related to their in vitro antioxidative activity. It is known that some anthocyanins have a cholesterol-lowering effect,4,6 anti-mutagenic and anti-tumor effects,7,8 an inhibitory effect on platelet aggregation,9 an anti-ulcer effect,10 and a promoting effect on rhodopsin synthesis.11 It is also known that red cabbage anthocyanins, which are used as food colorants, consist of many kinds of acylated anthocyanins12,13 and have antioxidative activity in vitro.14 However, the physiological functions of red cabbage (Brassica oleracea L.) acylated anthocyanins in vivo have not previously been reported.

In this study, the preventive effects of acylated anthocyanin (AcAnt) of red cabbage against the oxidative stress induced by paraquat (PQ), which is known to generate active oxygen species such as the superoxide radical and hydrogen peroxide through a redox cycling reaction,15 were determined in rats. Although the preventive effects of AnAnt against PQ-induced oxidative stress could possibly be determined by administering PQ to rats that had previously been given an AcAnt-added diet, instead a diet with PQ, AcAnt, or both PQ and AcAnt was used for this experiment, because almost all PQ and AcAnt in the diets remained without change during the daily feeding period, and because AcAnt that may be simultaneously absorbed with PQ can be expected to scavenge PQ-derived radicals at an early stage.

Materials and Methods

Preparation of acylated anthocyanin. Anthocyanins of red cabbage were extracted with a 3% trifluoro-acetic acid solution at 5°C and then filtered. The extract was passed through a column of XAD-7 (Organo Co., Tokyo, Japan). After washing the column with H2O, the anthocyanins were successively

† To whom correspondence should be addressed. Fax: +81-235-28-2928; E-mail: igarashi@tds1.tr.yamagata-u.ac.jp
eluted with 30%, 50% and 100% MeOH, and the 50% MeOH eluate was evaporated. The resulting residue, which was dissolved in a small amount of 0.01% HCl-MeOH, was precipitated by adding diethyl ether. The precipitate was dried in vacuo and dissolved in a small amount of solvent A (88% HCOOH–AcOH–H₂O = 1:1:9, v/v/v) for preparative HPLC. Preparative HPLC was carried out in a preparative ODS column (Develosil ODS HG-5, 20 × 250 mm, Nomura Chemical Co., Nagoya, Japan), using a linear gradient of 0–100% solvent B (88% HCOOH–AcOH–H₂O–MeOH = 1:1:6:3, v/v/v) in solvent A over the course of 80 min at a flow rate of 2.5 ml/min, and then by isocratic elution with solvent B. The fractions showing major peaks a, b and c on the chromatogram developed with the solvent system described in the legend to Fig. 2 were collected and evaporated. The residue was dissolved in a small amount of 0.01% HCl-MeOH and precipitated by adding diethyl ether. The precipitate obtained by this method was used for the animal experiments as acylated anthocyanins (AcAnt). On the other hand, compounds a, b and c on the HPLC chromatogram were further purified by Sephadex LH-20 column chromatography, using MeOH–AcOH–H₂O = 10:1:9 (v/v/v) as the eluent. Compounds a, b and c, which had been collected from the column, were evaporated to dryness, and their ¹H- and ¹³C-NMR and 2D NMR spectra, and their MS data were measured. FABMS measurement of a, b and c showed molecular ions at m/z 919, 949 and 973, respectively. The ¹H-NMR spectra of a, b and c were in good agreement with those of 3-O-(6-O-p-coumaryl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin, 3-O-(6-O-ferulyl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin, and 3-O-(6-O-sinapyl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin, respectively (Fig. 1).² The purity of AcAnt were about 91%, and the percentage amounts of a, b and c in AcAnt was about 42%, 22% and 27%, respectively when judged from the peak area of these compounds on the HPLC chromatogram (Fig. 2). The total yield of a, b and c was about 120 mg from 1 kg of fresh red cabbage.

**Fig. 1.** Chemical Structures of the Acylated Anthocyanins Used for the Animal Experiments.

a: R₁ = R₂ = OCH₃; 3-O-(6-O-sinapyl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin. b: R₁ = OCH₃, R₂ = H; 3-O-(6-O-ferulyl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin. c: R₁ = R₂ = H; 3-O-(6-O-p-coumaryl-2-O-(β-D-glucopyranosyl)-β-D-glucopyranosyl)-5-O-(β-D-glucopyranosyl) cyanidin

**Fig. 2.** Reversed-phase HPLC Analysis of the Acylated Anthocyanins (AcAnt) Used for the Animal Experiments.

AcAnt was analyzed in a ODS column (Nomura Chemical Co., Nagoya, Japan; Develosil C-30-UG-5, 4.6 × 250 mm), using a linear gradient of 0–100% of solvent B (88% HCOOH–AcOH–H₂O–CH₃CN = 0.5:0.5:2:0.1, v/v/v) in solvent A (88% HCOOH–AcOH–H₂O = 1:1:9, v/v/v) over the course of 120 min at a flow rate of 0.8 ml/min and subsequent isocratic elution with solvent B. Detection at 534 nm.

**Animals and diets.** Male weanling Wistar-strain rats, 4–5 weeks-old and each weighing about 55 g, were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan) and fed on a commercial diet (F-2, Funahashi Farms, Funahashi, Japan) for 2 d before being used in the experiment. They were randomly divided into four groups of 6 rats each for feeding on the basal diet and 5 rats each for the other three diets. The rats were individually housed in stainless-steel cages with screen bottoms and kept under controlled conditions with a 12-h light-dark cycle (06:00–18:00 light), a temperature range of 22–24°C, and a relative humidity of about 55%.

The compositions of the experimental diets are given in Table 1. The basal diet contained 20% casein without either paraquat (PQ) or AcAnt. The PQ diet was prepared by adding PQ (methyl viologen, Sigma Chemical Co., St. Louis, U.S.A.) to the basal diet at the 0.025% level. The AcAnt diet without PQ was prepared by adding AcAnt to the basal diet at the 0.15% level. The PQ + AcAnt diet was prepared by adding both PQ and AcAnt (at 0.025% and 0.15% levels, respectively) to the basal diet. The total amounts of the PQ, AcAnt and PQ + AcAnt diets
were made up to 100% by adjusting the amount of the mixture of α-corn starch and sucrose (2:1) that was added to the basal diet. PQ and AcAnt were added to the basal diet just before it was given to the rats. The amounts of PQ and AcAnt in the diet remained almost unchanged during the feeding period of 18 h, the percentage recovery of PQ and AcAnt from feed that had been left for 18 h under the feeding conditions being 94–96%. The amount of PQ in the supernatant, which was obtained by centrifuging a mixture of the diet and 2% sulfosalicylic acid, was determined by HPLC according to the method of Kage et al.\(^{10}\) with a Develosil C30-UG-5 column (4.6 × 250 mm, Nomura Chemical Co., Nagoya, Japan). The amount of AcAnt in the supernatant, which was obtained by centrifuging a mixture of the diet and 0.1% HCl-MeOH, was determined by measuring the OD value at 534 nm.

The amount of PQ added to the basal diet was that defined as the maximum non-toxic level for a rat (250 mg per kg of diet) by a committee from FAO and WHO.\(^ {17}\) Food and water were provided ad libitum for 10 d.

During the experiment, the rats were cared for according to the institutional guidelines of Yamagata University.

**Collection and preparation of blood and liver.** Blood was collected by cardiac puncture from rats that had been anesthetized with Nembutal (50 mg/ml; Dainippon Pharmaceutical Co., Osaka, Japan) after 10 h of starvation (from 03:00–07:00 to 13:00–17:00) at the end of the feeding period. A 0.1-ml aliquot of the blood was added to 1.9 ml of physiological saline while gently shaking, and the mixture was centrifuged at 1000 × g for 10 min to obtain serum for measuring the serum TBARS concentration and to provide erythrocytes. The erythrocytes were lysed with H₂O to measure the CuZn-superoxide dismutate (CuZn-SOD), catalase, and glutathione peroxidase (GSH-Px) activities.\(^ {10}\) A section from the right lobe of the liver, which had been excised from each of the rats, was used to measure the liver CuZn- and Mn-SOD, GSH-Px, catalase, glutathione reductase (GSSG-R), and NADPH-cytochrome P450-reductase activities. The other parts of the liver were stored at −30°C until needed for measuring the lipid and TBARS concentrations. Centrifuging the blood at 1000 × g for 15 min separated the serum for measuring the lipids.

**Preparation of the subcellular fraction.** It is known that PQ administration to mice or rats affects the antioxidant enzyme activities and TBARS level in both the liver and lung,\(^ {19–21}\) so the liver antioxidative enzyme activities and TBARS levels were measured in this experiment. The supernatant for measuring the antioxidative enzyme activities in the liver was prepared according the method of Del Boccio et al.\(^{22}\) A 0.5-g amount of the liver right lobe was homogenized in 2.5 ml of an ice-cold 0.1 m phosphate buffer (pH 7.4) containing 1 mm EDTA with a teflon pestle, and the homogenate was mixed with 2 volumes of 2.3% KCl, before being centrifuged at 600 × g for 3 min at 4°C to remove the fibrous material. The supernatant that had been obtained was further centrifuged at 1400 × g for 10 min at 4°C to remove the cellular debris. An aliquot of this supernatant was subsequently sonicated at 30 W for 2 min (four times for 30 sec each) by a Bransonics 1200 sonicator (Yamato Co., Tokyo, Japan). This sample was then centrifuged at 10,000 × g for 30 min at 4°C to obtain the mitochondrial fraction as a precipitate. This precipitate was sonicated in a 0.1 m phosphate buffer (pH 7.8), and the SOD and catalase activities were then measured. An aliquot of the supernatant which had been separated from the mitochondrial fraction was centrifuged at 105,000 × g for 60 min at 4°C to obtain the cytosolic fraction for measuring the SOD, catalase, GSH-Px and GSSG-R activities, and to obtain the microsomal fraction as a precipitate for measuring the NADPH-cytochrome P450 reductase activity.

SOD activity was measured by the xanthine-xanthine oxidase-nitroblue tetrazolium (NBT) method as described by Imanari et al.,\(^ {23}\) and catalase activity

---

Table 1. Composition of the Diets (%)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Con</th>
<th>+ PQ</th>
<th>+ AcAnt</th>
<th>+ AcAnt + PQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>α-Corn starch: sucrose = 2:1</td>
<td>65.5</td>
<td>65.475</td>
<td>65.35</td>
<td>65.325</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin mixture(^1)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture(^2)</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Paraquat</td>
<td>—</td>
<td>0.025</td>
<td>—</td>
<td>0.025</td>
</tr>
<tr>
<td>AcAnt(^3)</td>
<td>—</td>
<td>—</td>
<td>0.15</td>
<td>0.15</td>
</tr>
</tbody>
</table>

\(^1\) AIN-93-VX\(^{10}\) and \(^2\) AIN-93G-MX\(^{10}\) were obtained from Oriental Yeast Co. (Tokyo, Japan). AcAnt: acetylated anthocyanins.

\(^3\) Mixture of 3-O-[(6-O-sinapyl-2-O(β-D-glucopyranosyl)-β-D-glucopyranosyl)]-5-O-(β-D-glucopyranosyl) cyanidin, 3-O-[(6-O-coumaryl-2-O(β-D-glucopyranosyl)-β-D-glucopyranosyl)]-5-O-(β-D-glucopyranosyl) cyanidin, and 3-O-[6-O-p-coumaryl-2-O(β-D-glucopyranosyl)-β-D-glucopyranosyl]-5-O-(β-D-glucopyranosyl) cyanidin.
was measured by the spectrophotometric method to monitor the decrease in absorbance at 240 nm and 25°C due to hydrogen peroxide decomposition as described by Tomita et al. 20 The GSH-Px activity was measured by monitoring the decrease in absorbance of NADPH at 340 nm 21 by using 2-lutyl hydroperoxide as a substrate. The GSSG-R (glutathione reductase) activity was also measured by monitoring the decrease in NADPH, but by using oxidized glutathione as the substrate. 20 The definition of one unit of SOD and catalase activity is the amount of enzyme required to inhibit the rate of diironmisan formation from NBT by 50% per mg of hemoglobin or protein, and by the amount of enzyme to decompose 1 μmol of H₂O₂ per min per mg of hemoglobin or protein, respectively. The definition of one unit of GSH-Px and GSSG-R activity is the amount of enzyme required to oxidize 1 μmol of NADPH per min per mg of hemoglobin or protein, respectively.

The NADPH-cytochrome P450-reductase activity of the microsomal fraction, which had been stored at –30°C until required for analysis, was measured by using cytochrome C as the substrate, the time-course relationship for the decrease in absorbance at 550 nm being measured. 27 The definition of one unit of NADPH-cytochrome P450-reductase activity is the amount of enzyme required to reduce 1 μmol of cytochrome C per min per mg of protein.

The hemoglobin and protein contents were respectively measured by the method of Oshiro et al. 28 with a commercial hemoglobin test kit (Wako Pure Chemical Ind., Osaka, Japan) and by the method of Lowry et al., 29 using bovine serum albumin as the standard.

Measurement of TBARS. The serum TBARS concentration was determined by the method of Yagi 30 and is expressed as nmol of malondialdehyde per ml of blood. The liver TBARS concentration was measured by the method of Uchiyama and Mihrara, 31 using the homogenate which had been obtained by homogenizing 0.5 g of the liver with 2.5 ml of an ice-cold 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA as already described.

Lipid analyses. Serum total cholesterol was determined by the cholesterol oxidase method 32 with a cholesterol E-test kit (Wako Pure Chemical Ind.), triacylglycerol by the glycerol-3-phosphate oxidase method 33 with a triglyceride E-test kit (Wako Pure Chemical Ind.), and phospholipid by the choline oxidase method 34 with a phospholipid B-test kit (Wako Pure Chemical Ind.). Serum HDL-cholesterol was enzymatically measured in the supernatant obtained after heparin-Mn precipitation of the other lipoproteins. 35

The lipids of the frozen livers were extracted and purified by the method of Folch et al. 36 The lipids in the extract were measured by the same methods as those used for the serum, with the exception that total cholesterol was determined by the cholesterol oxidase method 37 with a Mono-test cholesterol kit (Boehringer Mannheim Yamanouchi Co., Tokyo, Japan). The atherogenic index is expressed as (total cholesterol – HDL-cholesterol)/HDL-cholesterol.

Statistical analyses. Data from 6 rats in the basal diet group and from 5 rats in each of the PQ, AcAnt and PQ + AcAnt diet groups were used for statistical analyses. The data for each of the four groups were statistically analyzed by Duncan’s multiple-range test 38 after a two-way analysis of variance (ANOVA), with the exception that the food intake and body weight were compared by applying the Mann-Whitney test for nonparametric data. Significant differences in the means were inspected at P < 0.05. A tendency of increase or decrease in the mean value of one group, when compared to another group, was inspected at 0.05 < P < 0.1.

Results

Food intake, and body and organ weights
As shown in Fig. 3, the food intake and body weight of the rats fed on the PQ diet (PQ group) each underwent a statistically significant decrease after 7 and 8 d when compared with the figure for the control rats (basal diet-fed rats). Both the lung weight and relative weight of the lung to the body weight were increased by PQ. The liver weight decreased in the PQ-fed rats when compared with that of the control rats, but the relative weight of the liver did not (Table 2). The supplement of AcAnt to the PQ diet relieved the decreased food intake and body weight, and the increased lung weight. An interaction between PQ and AcAnt was noted in the food intake, body weight gain, and lung weight (Table 2).

TBARS and antioxidative enzyme activities
As shown in Table 2, the serum TBARS concentration did not differ among the 4 groups. The TBARS concentration in the liver lipids of the PQ-fed rats was significantly higher than that of the control rats, but the liver TBARS concentration expressed as nmol/g of liver did not differ among the 4 groups. The addition of AcAnt to the PQ diet tended to relieve the increase in TBARS concentration in the liver lipid.

The SOD activity in the erythrocytes was significantly decreased by feeding the PQ diet, and an effect of PQ was observed on the SOD activity. The catalase and GSH-Px activities in the erythrocytes were not affected by PQ and AcAnt. The catalase activity in the liver mitochondrial fraction was markedly lower in the PQ-fed rats, but was partially recovered by supplementing the PQ diet with AcAnt. The
SOD, catalase, GSH-Px and GSSG-R activities in the liver cytosolic fraction did not show any statistically significant differences among the 4 groups.

An increase in the NADPH-cytochrome-P450 reductase activity by feeding the PQ diet was relieved by supplementing AcAnt to the PQ diet, and an effect of PQ, and interaction between PQ and AcAnt were apparent.

**Lipid levels**

Table 3 shows that the atherogenic index was increased to a statistically significant level in the PQ-fed rats when compared to that in the control rats. The supplement of AcAnt to the PQ diet significantly suppressed this increase in the atherogenic index. The concentrations of serum total cholesterol and triacylglycerol were not affected by dietary manipulation.

The concentration of liver triacylglycerol was significantly decreased in the PQ-fed rats, when compared to the level of the control rats, and was partially returned to the level of the control rats by supplementing AcAnt to the PQ diet. The other lipid concentrations were no different between the PQ and PQ + AcAnt groups.

**Discussion**

The respective prevention by AcAnt of the decreased food intake and body weight after feeding for 7 and 8 d, and of the increased lung weight and decreased liver weight at the end of the feeding period indicates the possibility that AcAnt could act preventively against PQ toxicity which is known to induce oxidative damage to such organs as the lungs, liver, kidneys and heart.20,39 The decreased food intake in the PQ-fed rats after 7 d may indicate that biochemical markers such as the antioxidative enzyme activities may have begun to change on day 7. However, it is necessary to further examine the time-course relationship for the changes in biochemical markers by administering PQ.

The serum TBARS concentration was not significantly affected by feeding the PQ diet or by supplementing AcAnt to the PQ diet. However, the concentration of TBARS in the liver lipids, which was increased by the PQ diet, tended to be decreased by supplementing AcAnt to the PQ diet. These results indicate the possibility that AcAnt can suppress the increase in liver lipid peroxidation due to PQ. When the concentration of liver TBARS is expressed as nmol/g of liver, the value for the PQ-fed rats did not differ from that for the rats fed on the PQ diet supplemented with AcAnt. This result indicates that it may be necessary to investigate the protective effects of AcAnt against oxidative stress by measuring another indicator for oxidative stress such as the ratio of oxidized glutathione to reduced glutathione.40 However, the suppression by AcAnt of decreased body weight gain, and of increased lung weight and TBARS in the liver lipids which were induced by PQ may support the possibility that AcAnt could act antioxidatively in vivo.

The GSH-Px and catalase activities in the erythrocytes, and the SOD, catalase, GSH-Px and GSSG-R activities in the liver cytosolic fraction were not affected by either PQ or AcAnt. However, the SOD activity in the erythrocytes and the catalase activity in the mitochondrial fraction were lower in the PQ-fed rats than in the control rats. Catalase in the mitochondrial fraction appears to have been the enzyme susceptible to PQ-induced oxidative stress. However the precise mechanism for the decrease in catalase activity by PQ in the mitochondrial fraction remains to be further examined. The suppression of catalase activity by PQ would further aggravate the oxidative damage by PQ. As the daily food intake of the PQ-fed rats was a little less than that of the rats fed on the PQ diet supplemented with AcAnt, it may be necessary to investigate by the pair-feeding
Table 2. Effects of Acetylated Anthocyanins (AcAnt) on the Food Intake, Body Weight Gain, Liver Weight, Lung Weight, and Antioxidative Status in Rats Fed on a Diet with or without Paraquat

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>+ PQ</th>
<th>+ AcAnt</th>
<th>+ PQ + AcAnt</th>
<th>Effect of PQ</th>
<th>Effect of AcAnt</th>
<th>Interaction between PQ and AcAnt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>61.2 ± 1.5</td>
<td>61.4 ± 1.0</td>
<td>61.2 ± 1.8</td>
<td>61.9 ± 0.9</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Food intake (g/10 days)</td>
<td>100 ± 0.33</td>
<td>73.2 ± 3.0</td>
<td>99.7 ± 0.46</td>
<td>25.1 ± 2.0</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Body weight gain (g/10 days)</td>
<td>36.8 ± 1.00</td>
<td>31.6 ± 2.3</td>
<td>37.3 ± 1.00</td>
<td>25.1 ± 2.0</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>3.7 ± 0.18</td>
<td>3.7 ± 0.13</td>
<td>3.8 ± 0.14</td>
<td>3.05 ± 0.12</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Liver weight (% of body weight)</td>
<td>3.9 ± 0.20</td>
<td>3.7 ± 0.17</td>
<td>3.7 ± 0.34</td>
<td>3.51 ± 0.12</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>0.60 ± 0.021</td>
<td>0.79 ± 0.057</td>
<td>0.60 ± 0.018</td>
<td>0.696 ± 0.012</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Lung weight (% of body weight)</td>
<td>0.614 ± 0.020</td>
<td>1.09 ± 0.10</td>
<td>0.610 ± 0.017</td>
<td>0.801 ± 0.021</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Serum TBARS (mmol/ml of blood)</td>
<td>1.59 ± 0.16</td>
<td>2.11 ± 0.19</td>
<td>1.99 ± 0.16</td>
<td>1.91 ± 0.08</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Liver TBARS (mmol/g of liver)</td>
<td>31.5 ± 0.8</td>
<td>30.5 ± 1.7</td>
<td>32.7 ± 2.5</td>
<td>30.4 ± 1.0</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Liver TBARS (mmol/mg of lipid)</td>
<td>0.444 ± 0.027</td>
<td>0.885 ± 0.034</td>
<td>0.500 ± 0.040</td>
<td>0.778 ± 0.033</td>
<td>P &gt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Antioxidative enzyme activity

Table 3. Effect of Acetylated Anthocyanins (AcAnt) on the Serum and Liver Lipid Concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>+ PQ</th>
<th>+ AcAnt</th>
<th>+ PQ + AcAnt</th>
<th>Effect of PQ</th>
<th>Effect of AcAnt</th>
<th>Interaction between PQ and AcAnt</th>
</tr>
</thead>
<tbody>
<tr>
<td>in the serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>1.74 ± 0.29</td>
<td>2.11 ± 0.13</td>
<td>1.67 ± 0.06</td>
<td>1.69 ± 0.13</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.50 ± 0.25</td>
<td>1.49 ± 0.20</td>
<td>1.43 ± 0.05</td>
<td>1.38 ± 0.10</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>0.166 ± 0.023</td>
<td>0.435 ± 0.083</td>
<td>0.170 ± 0.018</td>
<td>0.224 ± 0.016</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.02</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>0.308 ± 0.054</td>
<td>0.283 ± 0.068</td>
<td>0.301 ± 0.022</td>
<td>0.300 ± 0.034</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phospholipid (mmol/l)</td>
<td>1.31 ± 0.15</td>
<td>1.34 ± 0.15</td>
<td>1.25 ± 0.04</td>
<td>1.24 ± 0.11</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>in the liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/g of liver)</td>
<td>9.24 ± 0.49</td>
<td>6.85 ± 0.45</td>
<td>8.72 ± 0.61</td>
<td>6.61 ± 0.54</td>
<td>P &gt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/g of liver)</td>
<td>53.7 ± 4.2</td>
<td>46.4 ± 1.19</td>
<td>47.2 ± 3.9</td>
<td>11.6 ± 1.5</td>
<td>P &gt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Phospholipid (mmol/l)</td>
<td>17.3 ± 0.5</td>
<td>17.1 ± 0.7</td>
<td>17.6 ± 0.4</td>
<td>16.4 ± 0.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Each value is the mean ± S.E. for 5–6 rats per group. Values within the same row and not sharing a common superscript are significantly different at P < 0.05. Values without a superscript in the same row are not statistically different.

Method whether or not the decreased liver catalase activity by PQ was due to PQ toxicity. However, the decreased catalase activity in the rats with decreased body weight by PQ feeding may have been due to PQ toxicity, because it has been reported that the liver catalase activity increased in rats with decreased body weight when feeding on a restricted diet in comparison to that in rats fed on an ad libitum diet. The suppressed decrease in catalase activity by supplementing AcAnt to the PQ diet may suggest that AcAnt can prevent damage to catalase which may be caused by active oxygen species generated by paraquat redox cycling in the cells.

The suppression by AcAnt of the increase in atherogenic index due to PQ suggests that AcAnt may be effective in preventing the development of atherosclerosis due to oxidative stress. Although it has been reported that severe oxidative stress by PQ induced an increase in the serum cholesterol level, there are no reports on an increase in the atherogenic index by PQ, except for that in our previous work. The increase in atherogenic index in the PQ-fed rats may have been mainly due to an increase in VLDL+LDL cholesterol by PQ, because the HDL...
cholesterol level was hardly unchanged by PQ feeding. One reason for the decrease in liver triacylglycerol in the PQ-fed rats may have been due to its use as an energy source by the animals which could not retain their body weight after 8 d of the feeding period.

It has been reported that some anthocyanins showed antioxidative activity *in vitro*, and could prevent the oxidation of LDL *in vitro*. However, the antioxidative activity of AcAn *in vivo* has not been studied before. The results of this study suggest that AcAn could act antioxidatively and prevent oxidative stress *in vivo*. As it has recently been demonstrated that such anthocyanins as cyanidin-3-glucoside and cyanidin-3,5-diglucoside were absorbed from the digestive tract, some amount of AcAn given to rats as a food component is considered to have been absorbed from the digestive tract. However, it is necessary to precisely examine whether AcAn and/or its metabolites are absorbed or not and whether the absorbed AcAn and/or its metabolites themselves act antioxidatively against PQ-induced oxidative stress *in vivo*. It may also be interesting to examine which compound in AcAn is the most effective of the three.

The amount of AcAn ingested from red cabbage or from food colorants per day may be small. However, the ingestion of AcAn over a long period may be useful to prevent oxidative stress, even if the amount of AcAn ingested per day is minimal.

**Acknowledgments**

This work was supported by grant-aid from the San-Ei Gen foundation for food chemical research, and in part by grant-aid for scientific research (project no. 09660129) from Ministry of Education and Culture of Japan.

**References**


17. 1970 Evaluation of some pesticide residues in food, the monographs. Issued jointly by FAO and WHO.


21. He, P., Yamaoka-Koseki, S., and Yasumoto, K., Effects of pararquet administration on antioxidative enzyme activities and oxidative damage in the blood.


