Increase of Antioxidative Potential of Rat Plasma by Oral Administration of Proanthocyanidin-Rich Extract from Grape Seeds

Takuro Koga,*,† Keiko Moro,† Kaoru Nakamori,‡ Jun Yamakoshi,‡ Hiroshi Hosoyama,‡ Shigehiro Kataoka,‡ and Toshiaki Ariga‡

Noda Institute for Scientific Research, 399 Noda, Noda-shi, Chiba 278, Japan, and Research and Development Division, Kikkoman Corporation, 399 Noda, Noda-shi, Chiba 278, Japan

The effect of a single oral administration of proanthocyanidins, oligomeric and polymeric polyhydroxyflavan-3-ol units, on the antioxidative potential of blood plasma was studied in rats. Proanthocyanidin-rich extract from grape seeds was administered by intragastric intubation to fasted rats at 250 mg/kg of body weight. The plasma obtained from water- or proanthocyanidin-administered rats was oxidized by incubation with copper sulfate or 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) at 37 °C, and the formation of cholesteryl ester hydroperoxides (CE-OOH) was followed. The plasma obtained from proanthocyanidin-administered rats was significantly more resistant against both copper ion-induced and AAPH-induced formation of CE-OOH than that from control rats. The lag phase in the copper ion-induced oxidation of rat plasma was remarkably increased at 15 min after administration of proanthocyanidins and reached a maximum level at 30 min. When the plasma from proanthocyanidin-administered rat was hydrolyzed by sulfatase and β-glucuronidase following analysis by high-performance liquid chromatography with electrochemical detection, metabolites of proanthocyanidins occurred in rat plasma at 15 min after administration, three peaks of which were identified as gallic acid, (+)-catechin, and (−)-epicatechin. These results suggest that the intake of proanthocyanidins, the major polyphenols in red wine, increases the resistance of blood plasma against oxidative stress and may contribute to physiological functions of plant food including wine through their in vivo antioxidative ability.

INTRODUCTION

Grape seeds are rich sources of phenolic compounds, in which the most abundant classes of flavonoids include the flavan-3-ols. Some of the compounds in the flavan-3-ol class are sometimes referred to as catechins, which comprise the monomeric compounds, (+)-catechin, and (−)-epicatechin, as well as epicatechin 3-*O*-gallate. Grape seeds also contain small amounts of gallocatechins. However, in most cases the largest proportions of flavan-3-ols are found not in the monomeric form but in oligomeric and polymeric forms (Waterhouse and Walzem, 1998). Proanthocyanidins are oligomers and polymers of polyhydroxyflavan-3-ol units.

Grape seeds contribute to the catechin and proanthocyanidin contents of red wine (Kovac et al., 1995). Recently, much attention has been focused on wine's polyphenolic fraction, mainly flavonoids, to explain the mechanism of the "French Paradox"; that is, wine consumption is correlated with a reduced incidence of cardiovascular disease (St. Leger et al., 1979; Renaud and De Lorgeril, 1992). It has been reported that flavonoids occurring in grape and red wine have potent antioxidant activity and protect low-density lipoprotein (LDL) against oxidation in vitro (Frankel et al., 1993, 1995; Lanningham-Foster et al., 1995; Teissedre et al.,

1996; Viana et al., 1996; Kerry and Abbey, 1997; Meyer et al., 1997; Ghiselli et al., 1998). Thus, it is attractive to speculate that the beneficial effects of wine on coronary artery disease result from antioxidant protection of LDL by flavonoids in wine. However, information on the absorption and metabolism of ingested flavonoids is very limited, and relatively little is known about the in vivo antioxidant activity of flavonoids, particularly their oligomeric forms such as proanthocyanidins. In the present study, we investigated the in vivo antioxidant effect of proanthocyanidin-rich extract from grape seeds as a constituent in red wine by measuring plasma antioxidative ability of rat after a single oral administration of the extract. The results strongly suggest that proanthocyanidins act as antioxidants in blood circulation even after absorption and may contribute to the physiological functions of plant foods including wine through their in vivo antioxidative ability.

MATERIALS AND METHODS

Chemicals. Proanthocyanidin-rich extract was prepared from grape seeds (*Vitis vinifera* L.), and the contents of proanthocyanidins and monomeric flavanols were measured as described previously (Saito et al., 1998). Briefly, grape seeds were washed with water at 60 °C for 2 h and then extracted with water at 90 °C for 2 h. The aqueous extract was freeze-dried to obtain proanthocyanidin-rich extract. The extract was composed of 73.4% proanthocyanidins, 5.6% monomeric flavanols, 6.4% organic acids, 3.9% ash, 3.7% protein, 3.0% moisture, and 1.7% carbohydrate. 2,2′-Azobis(2-amidinopro-

* Author to whom correspondence should be addressed (telephone 81-471-23-5569; fax 81-471-23-5550).
† Noda Institute for Scientific Research.
‡ Kikkoman Corp.

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pane) dihydrochloride (AAPH), butylated hydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol; BHT), and gallic acid were purchased from Wako Pure Chemical Co. (Osaka, Japan). D-α-Tocopherol was obtained from Eisai Co. (Tokyo, Japan). (+)-Catechin, (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate were purchased from Funakoshi Co. (Tokyo, Japan). Procyanidins B2 and B4 were synthesized according to the method of Ricardo da Silva et al. (1991b), whereas procyanidin B3 was prepared and purified by Sephadex LH-20 and preparative HPLC as described previously (Saito et al., 1998). All other chemicals were of analytical grade, and solvents were of HPLC grade.

Animals and Oral Administration of Proanthocyanidins. Seven-week-old male Wistar rats were obtained from Charles River Japan Inc. (Yokohama, Japan). All rats were kept in an air-conditioned room (23 ± 1 °C and 55 ± 5% humidity) under a 12-h dark/light cycle. They were given a polyphenol-free diet (Okushio et al., 1996) and water ad libitum for a week. All animals were fasted for 16−18 h before administration. Proanthocyanidins were dissolved in water at the concentration of 25 mg/mL and administered intragastrically by direct stomach intubation to rats at a dose of 250 mg/kg of body weight. In a control group, three rats were administered 2 mL of plain water. Three rats for each group were anesthetized with pentobarbital at a specific time after administration, and blood was drawn from the abdominal aorta into heparinized tubes. Plasma was separated by centrifugation at 1000*g* for 20 min at 4 °C and was stored at −80 °C until use, but for a period not exceeding 4 days.

Oxidation of Rat Plasma. Plasma (0.6 mL) was mixed with 1.7 mL of phosphate-buffered saline (PBS, pH 7.4) in a test tube. The oxidation was initiated by the addition of 100 µL of $CuSO_4$ (2.4 mM) or AAPH (120 mM) dissolved in water or PBS, respectively. The final mixture was incubated at 37 °C in a shaking water bath in the dark under air. At specific time intervals, an aliquot of the suspension (100 µL) was withdrawn, and 3 mL of methanol containing 2.5 mM BHT, which prevents oxidation during extraction, was added to the reaction mixture. The mixture was sonicated in an ultrasonicator (Branson, model B1210J-DTH, Yamato Co., Tokyo, Japan) for 1 min, and then *n*-hexane (3 mL) was added to the mixture followed by centrifugation at 1500*g* for 3 min. Hexane layers were collected two times after extraction and evaporated in a rotary evaporator. The residue was dissolved in a mixture of acetonitrile and chloroform (3:2, v/v, 100 µL) for HPLC analyses of cholesteryl ester hydroperoxides (CE-OOH) and α-tocopherol.

Measurement of CE-OOH. CE-OOH was measured by HPLC using a TSK gel Octyl-80 Ts column (4.6 × 100 mm; Tosoh, Tokyo Japan). The mobile phase was composed of acetonitrile/chloroform (92.5:7.5, v/v) and used at a flow rate of 1.0 mL/min. CE-OOH were detected by ultraviolet absorption at 235 nm, and their concentrations were calculated from the standard curve of the hydroperoxy derivative of cholesteryl linoleate (Arai et al., 1997).

Measurement of α-Tocopherol. α-Tocopherol was measured by HPLC using a TSK gel Octyl 80Ts (4.6 × 100 mm, Tosoh). A mixture of acetonitrile and water (99:1, v/v) was used as mobile phase at a flow rate of 1.2 mL/min. The elute was monitored fluorometrically at an excitation wavelength of 298 nm and an emission wavelength of 325 nm using a Shimadzu RF-10A (Shimadzu Co., Kyoto, Japan). The concentration was calculated from the standard curve of the authentic compound.

Determination of Plasma Metabolites. The metabolites were extracted from plasma according to the method of Piskula and Terao (1998) with a slight modification. A plasma aliquot (100 µL) was mixed with 100 µL of 0.2 M acetate buffer (pH 5.0) containing sulfatase (25 units) and β-glucronidase (500 units) (sulfatase type H-5, which contains β-glucuronidase, Sigma Chemical Co., St. Louis, MO) and incubated at 37 °C in a shaking water bath for 45 min to hydrolyze conjugated metabolites into free forms. After hydrolysis, an aliquot of plasma (200 µL) was extracted with 800 µL of acetone/water/phosphoric acid (70:30:5, v/v/v). The mixture was vortexed for 1 min, sonicated for 30 s, and centrifuged for 5 min at 5000*g*.
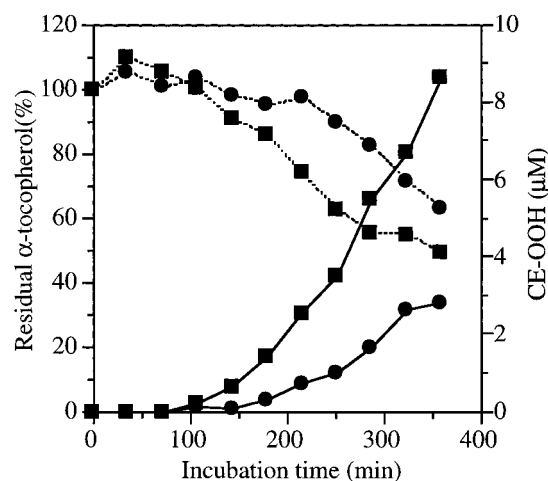

Figure 1. Accumulation of CE-OOH (solid line) and consumption of α-tocopherol (dotted line) in $CuSO_4$-induced oxidation of rat plasma obtained 1 h after oral administration of 2 mL of water or proanthocyanidins at 250 mg/kg of body weight: (■) control rat; (●) proanthocyanidin-administered rat. Plasma was diluted four times with PBS and incubated with $CuSO_4$ (100 µM) at 37 °C in the dark under air. The data represent several separate experiments.

The supernatant was evaporated in a rotary evaporator, and the residue was dissolved in 200 µL of acetonitrile/water (4:1, v/v). The aliquot (20 µL) was injected on an HPLC column (Senshu Pak Pegasil-B ODS, 6.0 × 250 mm, Senshu Kagaku Co., Tokyo, Japan) with a solvent flow of 0.8 mL/min. The solvents used for the separation were solvent A, 0.5% phosphoric acid, and solvent B, acetonitrile. The solvent gradient consisted of increasing the concentration of acetonitrile from 12% at start during 10 min to 33% at 50 min, followed by 33% for 30 min. The elute was monitored with amperometric detector (EC8020, Tosoh) with a working potential at +800 mV. The retention times of authentic compounds were 8.8 min for gallic acid, 18.4 min for (−)-epigallocatechin, 20.1 min for procyanidin B3, 24.0 min for (+)-catechin, 28.6 min for procyanidin B4, 30.2 min for procyanidin B2, 36.5 min for (−)-epicatechin, 39.0 min for epigallocatechin gallate, and 50.5 min for (−)-epicatechin gallate.

Statistical Analysis. Statistical analysis was tested by Student's *t* test.

RESULTS

Effect of Oral Administration of Proanthocyanidins on Copper Ion-Mediated Lipid Peroxidation of Rat Plasma. Plasma was obtained from rats at 1 h after a single oral administration of proanthocyanidins or water. Plasma was oxidized by adding 100 µM $CuSO_4$, and the formation of lipid hydroperoxides and the loss of α-tocopherol were measured. Figure 1 shows a typical example of the formation of CE-OOH and the consumption of α-tocopherol in the copper ion-induced oxidation of rat plasma. The formation of CE-OOH was started after the end of the clear lag phase. The lag phase, which is determined as the intercept of the baseline and propagation phase of the formation curve of CE-OOH, was significantly longer in the oxidation of plasma of proanthocyanidin-administered rats (166.8 ± 4.8 min) than that of control rats (100.8 ± 19.0 min). The level of CE-OOH formed during the oxidation of plasma was also higher in control rat plasma than in the plasma obtained from proanthocyanidin-administered rat. The plasma from proanthocyanidin-administered rats was more resistant against α-tocopherol consumption as compared with that from water-administered rat.
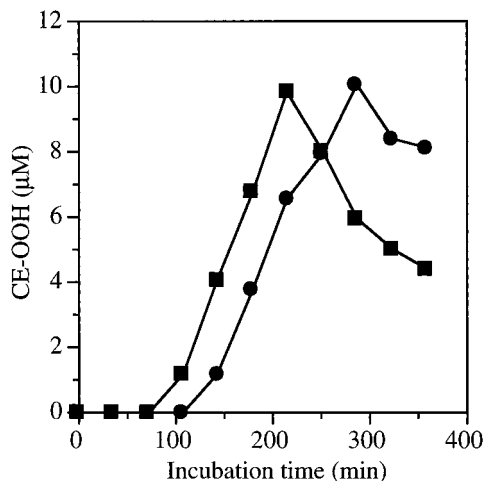
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Figure 2. Accumulation of CE-OOH in AAPH-induced oxidation of rat plasma obtained 1 h after oral administration of 2 mL of water or proanthocyanidins at 250 mg/kg of body weight: (■) control rat; (●) proanthocyanidin-administered rat. Plasma was diluted four times with PBS and incubated with AAPH (5 mM) at 37 °C in the dark under air. The data represent several separate experiments.

Effect of Oral Administration of Proanthocyanidins on AAPH-Mediated Lipid Peroxidation of Rat Plasma. When rat plasma was oxidized by incubation with free radical generator, AAPH (5 mM), the level of CE-OOH was linearly increased after the lag phase. The rat plasma obtained from proanthocyanidin-administered rats was more resistant against CE-OOH formation compared with control plasma (Figure 2). CE-OOH reached a maximum after 216 and 288 min of incubation in control and proanthocyanidin-administered rat plasma, respectively, and then decreased. In the case of AAPH-induced oxidation, the consumption of α-tocopherol was not followed because an unknown peak interfered with the peak corresponding to α-tocopherol in the HPLC analysis.

Effect of Oral Administration of Proanthocyanidins on the Lag Phase in Copper Ion-Mediated Lipid Peroxidation of Rat Plasma. Plasma obtained from rats before administration of proanthocyanidins displayed a lag phase of 96.8 ± 21.3 min when subjected to copper ion-mediated oxidation (Figure 3). Comparison of plasma obtained from proanthocyanidin-administered rats showed that the lag phase was significantly increased at 15 min after administration of proanthocyanidins. Rat plasma obtained at 480 min after administration of proanthocyanidins still gave a higher lag phase than that of control plasma.

HPLC Analysis of Plasma Metabolites in Proanthocyanidin-Administered Rats. When rat plasma was hydrolyzed by sulfatase and β-glucuronidase followed by HPLC analysis, many peaks were newly found in the HPLC chromatogram of plasma from proanthocyanidin-administered rat as compared with that of control plasma (Figure 4). Three peaks having retention times agreeing with those of the authentic standards were identified as gallic acid (8.83 min), (+)-catechin (24.03 min), and (−)-epicatechin (36.59 min). The levels of total metabolites of gallic acid, (+)-catechin, and (−)-epicatechin in plasma calculated from the standard curve of the authentic compounds were 9.2, 1.6, and 1.9 μM, respectively.
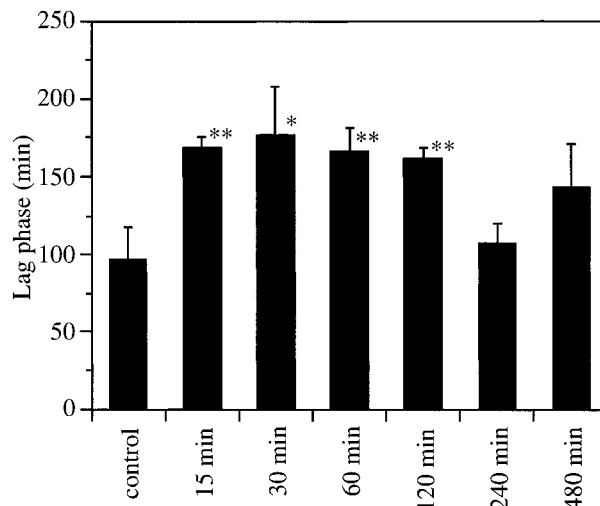

Figure 3. Effect of oral administration of proanthocyanidins on the lag phase of CE-OOH accumulation in CuSO₄-induced oxidation of rat plasma. Plasma was diluted four times with PBS and incubated with CuSO₄ (100 μM) at 37 °C in the dark under air. Bars represent the mean ± the standard deviation of three separate experiments. *, $P < 0.05$; **, $P < 0.01$.

DISCUSSION

Red wine contains numerous phenolic compounds, including flavonoids and non-flavonoids. Previous studies have shown that phenolic compounds in wine exert an in vitro antioxidative effect in plasma and LDL (Frankel et al., 1993, 1995; Lanningham-Foster et al., 1995; Teissedre et al., 1996; Viana et al., 1996; Kerry and Abbey, 1997; Meyer et al., 1997; Ghiselli et al., 1998). Oxidative modification of LDL is considered a key event in the development of atherosclerosis (Steinberg et al., 1989). Hence, the ability of wine phenolics to inhibit LDL oxidation has been suggested to be a possible mechanism explaining the "French Paradox". Proanthocyanidins, which are oligomers and polymers of polyhydroxy flavan-3-ol units such as (+)-catechin and (−)-epicatechin, and their gallate esters are present in wine as well as grape seeds (Lunte, 1987). Proanthocyanidins have remarkable radical scavenging activities (Ariga, 1985; Uchida et al., 1987; Ariga et al., 1988; Ariga and Hamano, 1990; Ricardo da Silva et al., 1991a) and inhibitory effect on LDL oxidation in vitro (Teissedre et al., 1996). However, there is little information on the in vivo antioxidant activity of proanthocyanidins. Here, we have demonstrated for the first time that orally administered proanthocyanidins do significantly increase the antioxidative defense in rat plasma.

In vivo studies showed that the antioxidative potential in humans responds to the oral ingestion of red wine (Kondo et al., 1994; Fuhrman et al., 1995; Whitehead et al., 1995) and the nonalcoholic fraction of red wine (Serafini et al., 1998). Thus, some substances that exist in red wine are absorbed and may be responsible for the in vivo antioxidant properties of red wine. Piskula and Terao (1998) reported that when (−)-epicatechin, which is commonly present in red wine and tea, was ingested by rats, it was absorbed and present in the blood circulation as various conjugated metabolites. Moreover, (−)-epicatechin metabolites possessed an effective antioxidant activity in blood plasma (Da Silva et al., 1998a). Our results showed that the antioxidant ability of rat plasma against both copper ion- and free radical generator-induced lipid peroxidation was remarkably increased after oral administration of proan-
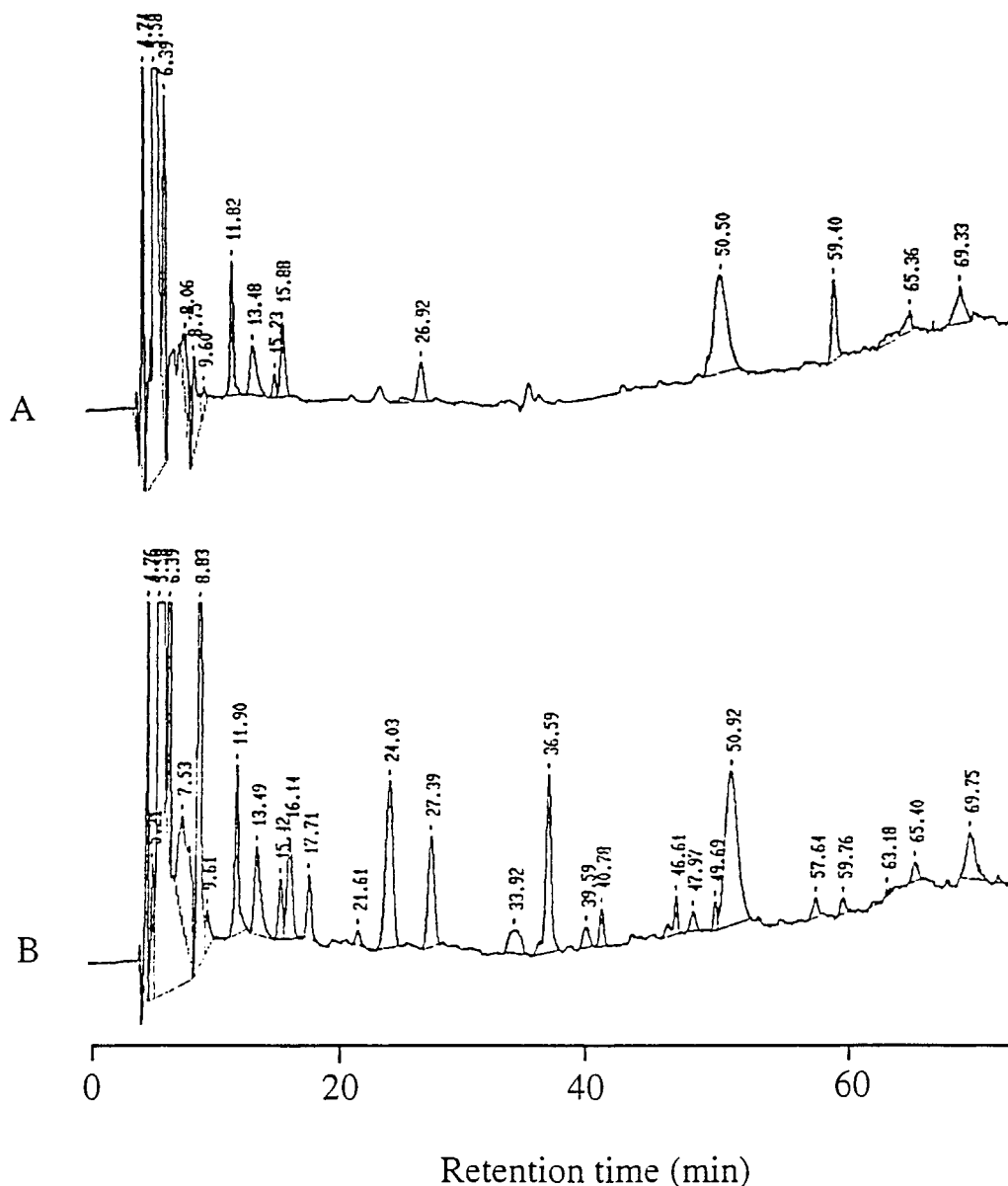
Figure 4. HPLC chromatograms of the extract from rat plasma after oral administration of water (A) or proanthocyanidins (B). Plasma was obtained 15 min after oral administration of 2 mL of water or proanthocyanidins at 250 mg/kg of body weight following the treatment with sulfatase and β-glucronidase to hydrolyze the conjugated metabolites.

thocyanidins and that their metabolites appeared in plasma. These results mean that flavonoids such as (−)-epicatechin and proanthocyanidins are absorbed and their plasma metabolites may act as antioxidants when reactive oxygen radicals are generated in blood circulation. It is therefore likely that these flavonoids are at least partly responsible for in vivo antioxidant properties of red wine.

In another paper, we have reported that feeding proanthocyanidins inhibited the progression of atherosclerosis in cholesterol-fed rabbits (Yamakoshi et al., 1999). We also found that plasma's ability to suppress lipid peroxidation was increased after 1 month of feeding of proanthocyanidins as compared to plasma collected from control rabbits. Thus, we can reasonably speculate that the antioxidative ability of proanthocyanidin metabolites found in plasma may be related to their antiatherosclerotic effect in cholesterol-fed rabbits. In fact, the decrease in the number of oxidized LDL positive macrophage-derived foam cells in atherosclerotic lesions was found in the aorta of rabbits fed

proanthocyanidins. Tebib et al. (1994, 1997) have reported that long-term dietary grape seed tannins, which contain proanthocyanidins, prevent an increase in total and LDL plasma cholesterol in high-cholesterol-fed rats and negate the loss of antioxidant potential in tissues of rats fed a high cholesterol—vitamin E-deficient diet. Recognition of these in vivo health benefits of proanthocyanidins has facilitated the use of grape seed extract containing proanthocyanidins as a dietary supplement. Plasma metabolites of proanthocyanidins seem to contribute to not only the rise of plasma antioxidative ability but also their physiological functions.

The time trend of plasma antioxidative potential after a single oral administration of proanthocyanidins showed that plasma antioxidative ability was remarkably increased at 15 min after oral administration of proanthocyanidins (Figure 3). Plasma metabolites of proanthocyanidins also appeared at 15 min after administration (Figure 4). These results suggest that the absorption occurs in the gastroduodenal region of fasted rats. Laparra et al. (1977) followed blood levels of radioactiv-

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ity in mice after a single oral administration of ^{14}C-labeled procyanidins and reported that their gastrointestinal absorption was rapid with a maximum level at 45 min. The appearance of flavonoid metabolites within 1 h after ingestion was also observed in (−)-epicatechin and quercetin in rats (Da Silva et al., 1998a,b), in epigallocatechin gallate in rats (Okushio et al., 1995; Unno and Takeo, 1995; Nakagawa and Miyazawa, 1997) and human (Lee et al., 1995), and in wine polyphenols in human (Serafini et al., 1998).

Following enzymatic treatment of plasma and HPLC determination, the metabolites appeared in the chromatogram of extracts from proanthocyanidin-administered rat plasma (Figure 4). The difference in the chromatogram after and before hydrolysis suggested that most compounds occur in blood circulation as glucuronide, sulfate, and glucuronide sulfate conjugates (data not shown). Most peaks have not been identified so far except for three peaks, which have been identified as gallic acid, (+)-catechin, and (−)-epicatechin from their retention times relative to the authentic standard. Because the content of monomeric polyphenols is only 5.6% in the grape seed extract used in this study, these metabolites may have originated mostly from the hydrolysis of proanthocyanidins in the digestive track of rat because gallic acid, (+)-catechin, and (−)-epicatechin found in plasma are major units in proanthocyanidins. Laparra et al. (1977) reported that when ^{14}C-labeled dimeric procyanidins were fed to mice, radioactivity was recovered in the plasma and various tissues. In another paper (Yamakoshi et al., 1999), we have reported that when Porter's method was applied to detect the oligomeric forms in proanthocyanidin-administered rat plasma, proanthocyanidins were detected at 18.1 ± 0.14 µg/mL. Therefore, it is likely that some unknown peaks appearing in the chromatogram may correspond to oligomeric forms such as dimer and trimer. Further investigation is necessary for characterization of the metabolic fate of proanthocyanidins.

CONCLUSION

Recent research (Serafini et al., 1997, 1998; Piskula and Terao, 1998; Da Silva et al., 1998a,b) and our findings suggest that flavonoids in plant foods and products, such as red wine and tea, are absorbed and may modulate red−ox status. From a nutritional aspect, metabolites of flavonoids should be taken into account, because dietary flavonoids are mostly converted to metabolites by conjugation and methylation, which might circulate in the blood stream. Therefore, it will be important to study the biological functions of flavonoids after absorption and metabolism.

ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; BHT, butylated hydroxytoluene; CE-OOH, cholesteryl ester hydroperoxides; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; PBS, phosphate-buffered saline.

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LITERATURE CITED

Arai, H.; Terao, J.; Abdalla, D. S. P.; Suzuki, T.; Takama, K. Coulometric detection in high-performance liquid chromatographic analysis of cholesteryl ester hydroperoxides. *Free Radical Biol. Med.* **1997**, *20*, 365−371.

Ariga, T. *Abstracts of Papers*, Annual Meeting of the Agricultural Chemical Society of Japan, Sapporo, 1985; p 282.

Ariga, T.; Hamano, M. Radical scavenging action and its mode in procyanidins B-1 and B-3 from Azuki beans to peroxyl radicals. *Agric. Biol. Chem.* **1990**, *54*, 2499−2504.

Ariga, T.; Koshiyama, I.; Fukushima, D. Antioxidative properties of procyanidins B-1 and B-3 from Azuki beans in aqueous systems. *Agric. Biol. Chem.* **1988**, *52*, 2717−2722.

Da Silva, E. L.; Piskula, M. K.; Terao, J. Enhancement of antioxidative ability of rat plasma by oral administration of (−)-epicatechin. *Free Radical Biol. Med.* **1998a**, *24*, 1209−1216.

Da Silva, E. L.; Piskula, M. K.; Yamamoto, N.; Moon, J.-H.; Terao, J. Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. *FEBS Lett.* **1998b**, *430*, 405−408.

Frankel, E. N.; Kanner, J.; German, J. B.; Parks, E.; Kinsella, J. E. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* **1993**, *341*, 454−457.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 880−894.

Fuhrman, B.; Lavy, A.; Aviram, M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoprotein to lipid peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549−554.

Ghiselli, A.; Nardini, M.; Baldi, A.; Scaccini, C. Antioxidant activity of different phenolic fractions separated from an Italian red wine. *J. Agric. Food Chem.* **1998**, *46*, 361−367.

Kerry, N. L.; Abbey, M. Red wine and fractionated phenolic compounds prepared from red wine inhibit low-density lipoproteine oxidation in vitro. *Atherosclerosis* **1997**, *135*, 93−102.

Kondo, K.; Matsumoto, A.; Kurata, H.; Tanahashi, H.; Koda, H.; Amachi, T.; Itakura, H. Inhibition of oxidation of low-density lipoprotein with red wine. *Lancet* **1994**, *344*, 1152.

Kovac, V.; Alonso, E.; Revilla, E. The effect of adding supplementary quantities of seeds during fermentation on the phenolic composition of wines. *Am. J. Enol. Vitic.* **1995**, *46*, 363−367.

Lanningham-Foster, L.; Chen, C.; Chance, D. S.; Loo, G. Grape extract inhibits lipid peroxidation of human low-density lipoprotein. *Biol. Pharm. Bull.* **1995**, *18*, 1347−1351.

Laparra, J.; Michaud, J.; Masquelier, J. Etude pharmacocinetique des oligomeres flavanoliques. *Plant. Med. Phytother.* **1977**, *11*, 133−142.

Lee, M.-J.; Wang, Z.-Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, G.; Balentine, D. A.; Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol. Biomarkers Prev.* **1995**, *4*, 393−399.

Lunte, S. M. Stractural classification of flavonoids in beverages by liquid chromatography with ultraviolet−visible and electrochemical detection. *J. Chromatgr.* **1987**, *384*, 371−382.

Meyer, A. S.; Yi, O.-S.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N. Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). *J. Agric. Food Chem.* **1997**, *45*, 1638−1643.

Nakagawa, K.; Miyazawa, T. Absorption and distribution of tea catechin, (−)-epigallocatechin-3-gallate, in the rat. *J. Nutr. Sci. Vitaminol.* **1997**, *43*, 679−684.

Okushio, K.; Matsumoto, N.; Suzuki, M.; Nanjo, F.; Hara, Y. Absorption of (−)-epigallocatechin gallate into rat portal vein. *Biol. Pharm. Bull.* **1995**, *18*, 190−191.

Antioxidant Activity of Grape Seed Extract in Rat Plasma

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Okushio, K.; Matsumoto, N.; Kohri, T.; Suzuki, M.; Nanjo, F.; Hara, Y. Absorption of tea catechins into rat portal vein. *Biol. Pharm. Bull.* **1996**, *19*, 326−329.

Piskula, M. K.; Terao, J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugated enzymes in rat tissues. *J. Nutr.* **1998**, *128*, 1172−1178.

Renaud, S.; De Lorgeril, M. Wine, alcohol, platelets, and the French Paradox for coronary heart disease. *Lancet* **1992**, *339*, 1523−1526.

Ricardo da Silva, J. M.; Darmon, N.; Fernandez, Y.; Mitjavila, S. Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. *J. Agric. Food Chem.* **1991a**, *39*, 1549−1552.

Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry* **1991b**, *30*, 1259−1264.

Saito, M.; Hosoyama, H.; Ariga, T.; Kataoka, S.; Yamaji, N. Antiulcer activity of grape seed extract and procyanidins. *J. Agric. Food Chem.* **1998**, *46*, 1460−1464.

Serafini, M.; Ghiselli, A.; Ferro-Luzzi, A. In vivo antioxidant effect of green tea and black tea in man. *Eur. J. Clin. Nutr.* **1997**, *50*, 28−32.

Serafini, M.; Maiani, G.; Ferro-Luzzi, A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. *J. Nutr.* **1998**, *128*, 1003−1007.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *New Engl. J. Med.* **1989**, *320*, 915−924.

St. Leger, A. S.; Moore, F.; Cochrane, A. L. Factors associated with cardiac mortality in developed countries with particular reference to consumption of wine. *Lancet* **1979**, *1*, 1017−1020.

Tebib, K.; Bitri, L.; Besancon, P.; Rouanet, J. M. Polymeric grape seed tannins prevent plasma cholesterol changes in high-cholesterol-fed rats. *Food Chem.* **1994**, *49*, 403−406.

Tebib, K.; Rouanet, J. M.; Besancon, P. Antioxidant effects of dietary polymeric grape seed tannins in tissues of rats fed a high cholesterol-vitamin E-deficient diet. *Food Chem.* **1997**, *59*, 135−141.

Teissedre, P. L.; Frankel, E. N.; Waterhouse, A. L.; Peleg, H.; German, J. B. Inhibition of in vitro human LDL oxidation by phenolic antioxidants from grapes and wines. *J. Sci. Food Agric.* **1996**, *70*, 55−61.

Uchida, S.; Edamatsu, R.; Hiramatsu, M.; Mori, A.; Nonaka, G. Y.; Nishioka, I.; Niwa, M.; Ozaki, M. Condensed tannins scavenge active oxygen free radicals. *Med. Sci. Res.* **1987**, *15*, 831−832.

Unno, T.; Takeo, T. Absorption of (−)-epigallocatechin gallate into the circulation system of rats. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1558−1559.

Viana, M.; Barbas, C.; Bonet, B.; Bonet, M. V.; Castro, M.; Fraile, M. V.; Herrera, E. In vitro effects of a flavonoid-rich extract on LDL oxidation. *Atherosclerosis* **1996**, *123*, 83−91.

Waterhouse, A. L.; Walzem, R. L. Nutrition of grape phenolics. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, L., Eds.; Dekker: New York, 1998; pp 359−385.

Whitehead, T. P.; Robinson, D.; Allaway, S.; Syms, J.; Hale, A. Effect of red wine ingestion on the antioxidant capacity of serum. *Clin. Chem.* **1995**, *41*, 32−35.

Yamakoshi, J.; Kataoka, S.; Koga, T.; Ariga, T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* **1999**, *142*, 139−149.