Orally Administered Delphinidin 3-Rutinoside and Cyanidin 3-Rutinoside Are Directly Absorbed in Rats and Humans and Appear in the Blood as the Intact Forms

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Four components of black currant anthocyanins (BCA), delphinidin 3-*O*-*β*-rutinoside (D3R), cyanidin 3-*O*-*β*-rutinoside (C3R), delphinidin 3-*O*-*β*-glucoside (D3G), and cyanidin 3-*O*-*β*-glucoside (C3G), were found to be directly absorbed and distributed to the blood and excreted into urine as the glycosylated forms. In a rat study, following oral administration of purified D3R, C3R, and C3G (800 *μ*mol/kg of body weight), the anthocyanins were detected in the plasma and the C_{max} values were 580 ± 410, 850 ± 120, and 840 ± 190 nmol/L, respectively, 0.5−2.0 h after administration. In a human study, when a mixtue of BCA [6.24 *μ*mol (3.58 mg) consisting of 2.75 *μ*mol (1.68 mg) of D3R, 2.08 *μ*mol (1.24 mg) of C3R, 1.04 *μ*mol (0.488 mg) of D3G, and 0.37 *μ*mol (0.165 mg) of C3G/kg of body weight)] was orally ingested by eight volunteers, D3R, C3R, D3G, and C3G were detected in the plasma and urine. The plasma C_{max} values were 73.4 ± 35.0, 46.3 ± 22.5, 22.7 ± 12.4, and 5.0 ± 3.7 nmol/L, respectively, 1.25−1.75 h after intake, and the cumulative excretion of the four compounds in urine in the period 0−8 h after intake was 0.11 ± 0.05% of the dose ingested. These results indicate that 3-*O*-*β*-rutinosyl anthocyanins were directly absorbed and distributed to the blood.

Keywords: *Anthocyanins; absorption; excretion; black currant; delphinidin 3-rutinoside; cyanidin 3-rutinoside; delphinidin 3-glucoside; cyanidin 3-glucoside; human; rat*

INTRODUCTION

Considerable amounts of anthocyanins are ingested as constituents of the human diet, ∼180−215 mg daily (*1*). Anthocyanins are present in fruits, beans, cereals, vegetables, and red wines. Moderate consumption of anthocyanins, through the intake of products such as red wine (*2*) or bilberry extract (*3*), is associated with a lower risk of coronary heart disease and improvement of visual functions. The health benefits of intake of anthocyanins are mainly attributable to their antioxidant, antiplatelet, ophthalmic, and vasoprotective activities, and these biochemical and physiological activities have attracted considerable research interest. Also, the bioavailability of anthocyanins in vivo including their absorption, metabolic fate, and excretion is viewed as an important issue.

Several studies on the absorption of flavonoid aglycons have been reported. However, there have been few studies on the absorption of glycosides, for example, naringin (*4*), quercetin glycosides (*5*), and luteolin glucosides (*6*). There have been only two reports of quantitative absorption studies using purified anthocyanins, cyanidin 3-*O*-*β*-glucoside (C3G) and cyanidin 3,5-di-*O*-glucoside (*7*, *8*), although a few studies have been performed using mixtures extracted from plant sources, such as *Vaccinium myrtillus* anthocyanosides (VMA) (*9*) and wine anthocyanins (*10*). There are no quantitative data available on the absorption of anthocyanin 3-*O*-rutinosides.

In our recent study, oral intake of a black currant anthocyanin (BCA) concentrate prepared from black currant (*Ribes nigrum* L.) juice was found to result in improvement of dark adaptation and transient alteration of vision induced by work at visual display terminals (VDT) in healthy humans (*11*). This finding strongly suggests that BCA is absorbed and that BCA and/or the metabolites formed in vivo display physiological activity. However, difficulties have been encountered in conducting quantitative absorption studies, as preparative-scale isolation of the individual components remains a challenge. It has been reported that C3G is absorbed in rats and humans, appearing in the blood along with its metabolites (*7*, *8*); however, there has been no report on the absorption of D3R or C3R, the two major anthocyanin 3-rutinoside components of BCA. With respect to studies on the absorption of flavonoid rutinosides in humans, there have been reports on only two compounds, rutin (quercetin 3-*O*-*β*-rutinoside) and naringin (naringenin 7-*O*-*β*-rutinoside). There was found to be a considerable difference in their absorption patterns in that the former showed markedly delayed absorption 6 h after intake (*5*), whereas the latter was rapidly excreted into urine within 2 h after intake (*12*).

Recently, we have succeeded in the preparative-scale isolation of four BCA components, delphinidin 3-*O*-*β*-rutinoside (D3R), cyanidin 3-*O*-*β*-rutinoside (C3R), delphinidin 3-*O*-*β*-glucoside (D3G), and cyanidin 3-*O*-*β*-glucoside (C3G) (*13*), and it became possible for us to

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conduct an absorption study and to analyze the kinetics quantitatively using these isolated compounds. The present paper describes the results of absorption studies in rats and humans. Each of the purified components (D3R, C3R, or C3G) was orally administered to rats to evaluate the absorption behavior. Also, a mixture of BCA (BCA concentrate) was orally ingested by healthy human subjects, and the absorption profiles of the four components were analyzed and compared with those observed in the case of rats.

MATERIALS AND METHODS

Chemicals. BCA concentrate and flavylium chlorides of three purified components, D3R, C3R, and C3G, were prepared from commercial black currant juice according to the methods described in a previous paper (*13*). The purity of the flavylium chlorides was >99.5%, and the structure of each was confirmed by UV, FAB-MS, and NMR spectra analyses. The total anthocyanin content of the BCA concentrate was 10.8%, consisting of D3R (5.08%), C3R (3.75%), D3G (1.48%), and C3G (0.50%). All other nutrients, reagents, and chemicals used were purchased from commercial sources.

Rat Study. Thirty-nine male Wistar rats at 6 weeks of age were obtained from Clea Japan Co., Ltd. (Tokyo; 75−90 g body weight), and individually housed in stainless steel wire-mesh cages at 23 ± 2 °C with a 12-h light−dark cycle. The animals were given free access to tap water and a semipurified diet. All rats were handled in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (*14*). For 1 week before the experiment, all animals were fed a control diet containing the following nutrients (g/kg of diet): cornstarch (532), casein (200), sucrose (100), corn oil (70), cellulose (50), mineral mixture (35), vitamin mixture (10) prepared according to the AIN-93G formulation, and L-cysteine (3). After the feeding period, food was withheld for 24 h. The rats were randomly assigned to three groups, and D3R, C3R, or C3G dissolved in water containing 0.1% citric acid was orally administered to the rats in the designated group by direct stomach intubation at a dosage of 800 μmol (489 mg of D3R, 476 mg of C3R, and 359 mg of C3G) of anthocyanin/kg of body weight (dosing volume = 1.7 ± 0.1 mL/rat). Three rats were killed at 0.5, 1.0, 2.0, and 4.0 h postadministration by withdrawing blood from the inferior vena cava using a heparinized needle and syringe under anesthesia with diethyl ether.

Human Study. Eight healthy male volunteers (60−72 kg body weight), between 26 and 57 years of age, participated in this study. The human study was performed according to the Helsinki Declaration. On the day before the experiment, the subjects did not consume any kind of food rich in anthocyanins (vegetables, fruit, juice, etc.). They did not ingest any food or beverages except for water in the 12-h period prior to the experiment. On the day of the experiment, a BCA concentrate [33.0 mg/kg of body weight, corresponding to 6.24 μmol (3.58 mg) as BCA, 2.75 μmol (1.68 mg) as D3R, 2.08 μmol (1.24 mg) as C3R, 1.04 μmol (0.488 mg) as D3G, and 0.37 μmol (0.165 mg) as C3G/kg body weight] in water (150 mL) was consumed orally. Blood samples were collected at 1.0, 2.0, 4.0, 6.0, and 8.0 h postingestion. During the experiment, the subjects drank 200 mL of water every 2 h for 8 h, and urine was collected in sterile tubes at 2-h intervals, 0−2, 2−4, 4−6, and 6−8 h after administration. Three hours after ingestion of the BCA concentrate, the subjects were served an anthocyanin-free lunch, consisting of only a rice ball (150 g) with salt.

Sample Preparation. Plasma samples were prepared according to the method of Tsuda et al. (*7*) with slight modification. In the case of both rat and human plasma, the plasma was immediately obtained from the collected blood by centrifugation at 1600*g* for 15 min at 4 °C. The plasma separation was completed within 30 min, and it was acidified with a $^1/_{40}$ volume of 6 N HCl. A portion of the rat plasma (400 μL) was diluted with 400 μL of 10 mmol/L oxalic acid. Each portion of the human plasma (4000 μL) and the diluted rat plasma (400 μL) was applied to Sep-Pak C_{18} cartridges (Waters, Milford, MA), which had been washed with 10 mL of methanol containing 5% TFA and equilibrated with 10 mL of 10 mmol/L oxalic acid before use. After washing with 10 mmol/L oxalic acid, anthocyanins were eluted with methanol containing 5% TFA, and the eluate was carefully evaporated to dryness in vacuo below 35 °C. The dried residue was redissolved in 200 μL of 3% phosphoric acid, and a portion (rat, 20 μL; human, 100 μL) of this solution was injected into an HPLC system for anthocyanin analysis. The method was validated by performing the following recovery test. Plasma D3R solutions (0.100 and 0.050 μmol/L) were prepared by adding D3R (0.26 and 0.13 μg) into anthocyanin-untreated rat plasma (400 μL), and the resultant solutions were treated in the same manner as those of anthocyanin-treated rats. Upon HPLC analysis of the extracts, the recoveries of D3R were found to be 72.7% for 0.100 μmol/L and 70.3% for 0.050 μmol/L plasma solutions.

A 1.0 mL portion of each urine sample was immediately used for measurement of the creatinine concentration. Other urine samples were acidified with a $^1/_{40}$ volume of 6 N HCl, and a portion (10 mL) was applied to a Sep-Pak plus C_{18} ENV cartridge, conditioned before use in the same manner as described for the plasma samples. After the cartridge had been washed with 10 mmol/L oxalic acid (10 mL), the adsorbed BCA were eluted with methanol (5.0 mL) containing 5% TFA. The eluate was evaporated in vacuo below 35 °C, and the resultant residue was redissolved in 3% phosphoric acid (200 μL). A portion (100 μL) of the solution was subjected to HPLC analysis.

Determination of Anthocyanins and Biomarkers. The identification and quantification of the four anthocyanins were performed using an HP 1100 series HPLC system equipped with a Zorbax SB C-18 column (4.6 mm × 250 mm, particle size = 5 mm) and a photodiode array detector at 520 nm (*13*). Injection was performed by means of an autosampler, with a 100 μL fixed loop. Elution was performed using a solvent system consisting of a mixture of solvent A (0.5% phosphoric acid) and solvent B (methanol), applied as a linear gradient from 80% A/20% B (v/v) to 77% A/23% B (v/v) for 15 min and then held at 77% A/23% B (v/v) for a further 8 min, at a flow rate of 1.0 mL/min. The eluted constituents were identified by measuring the photodiode array UV−vis spectra from 200 to 600 nm. The areas of the peaks of D3R, C3R, D3G, and C3G were proportional to the amounts injected, within the range of 0.2−400 ng, and the detection limit was 0.1 ng in each instance. The concentration of creatinine in urine was determined by means of a creatinine test kit (Wako Co., Ltd., Tokyo, Japan).

Pharmacokinetic Analysis. Analysis of the blood concentration−time data was performed by noncompartment model analysis using WinNonlin Professional (version 3.1, Pharsight Co., Mountain View, CA). The maximum plasma concentration (C_{max}), and the time to reach the maximum plasma concentration (t_{max}) was calculated from the observed values. The elimination rate constant (k_{el}) values were calculated from the blood level equation after curve-fitting, and the half-life ($t_{1/2}$) values were estimated as $(\ln 2)/k_{el}$. The total area under the concentration time curve [AUC (o→obs)] was calculated by the trapezoidal rule based on the plasma concentrations up to the time of final measurement.

RESULTS

Rat Study. After oral administration of purified D3R, C3R, or C3G, each at the same dosage (800 μmol/kg of body weight), the plasma concentrations were measured by HPLC. Figure 1 shows typical HPLC profiles of rat plasma 0.5 and 2.0 h after administration of the anthocyanins. D3R, C3R, and C3G were detected in the plasma as the intact forms. No other peaks were detected in the analysis of rat plasma after administration of D3R or C3R when the absorbance of the eluate was monitored at 520 nm (Figure 1A,B). However, upon analysis of plasma 2.0 h after the administration of

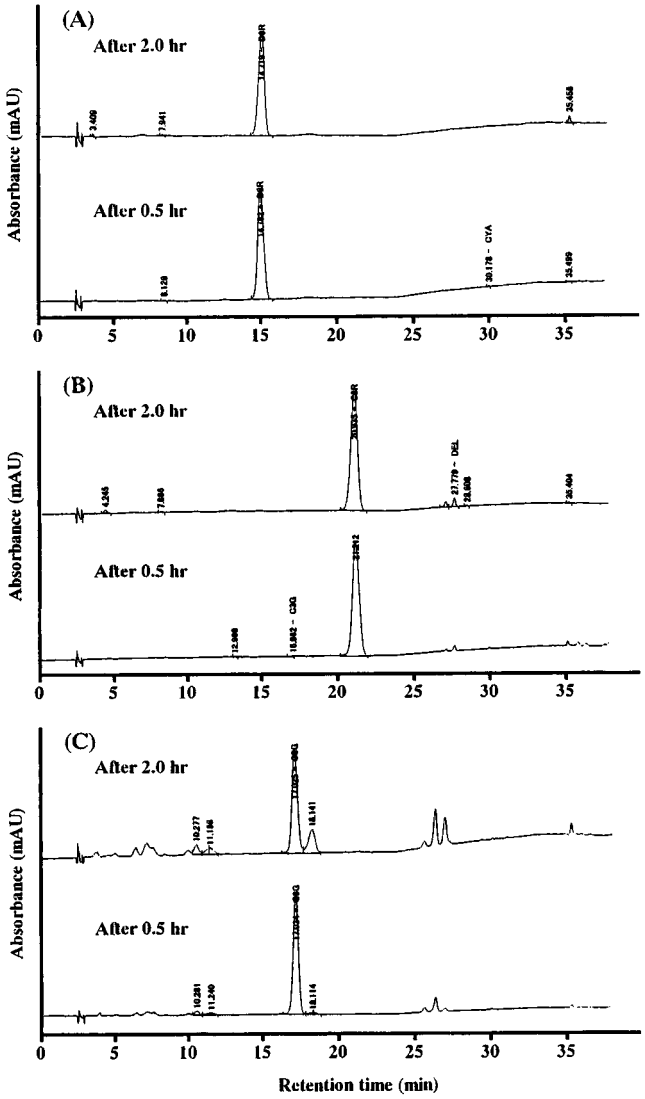

Figure 1. Typical HPLC chromatograms obtained in analysis of rat plasma 0.5 and 2.0 h after oral administration of purified anthocyanin (D3R, C3R, or C3G): (A) HPLC chromatogram obtained in analysis of rat plasma after administration of D3R; (B) HPLC chromatogram obtained in analysis of rat plasma after administration of C3R; (C) HPLC chromatogram obtained in analysis of rat plasma after administration of C3G. The eluted constituents were detected by monitoring the absorbance of the eluate at 520 nm.

C3G, two peaks were observed with retention times of 17.75 and 26.21 min. These peaks were significantly larger in area than those observed 0.5 h after administration. These peaks were assumed to be attributable to metabolites of C3G. The structural features of these compounds remain unknown.

Figure 2 shows the time course of changes in the concentrations of D3R, C3R, and C3G in rat plasma after the administration of these anthocyanins. None of these anthocyanins was detected in plasma before administration. Following oral administration of D3R, C3R, and C3G, there was a rapid increase in the plasma concentration to 230 ± 80, 850 ± 120, and 840 ± 190 nmol/L, respectively, at 0.5 h postadministration. The plasma concentration reached maximma of 580 ± 410 nmol/L at 2.0 h postadministration in the case of D3R, 850 ± 120 nmol/L at 0.5 h postadministration in the case of C3R, and 840 ± 190 nmol/L at 0.5 h postadministration in the case of C3G and then decreased.

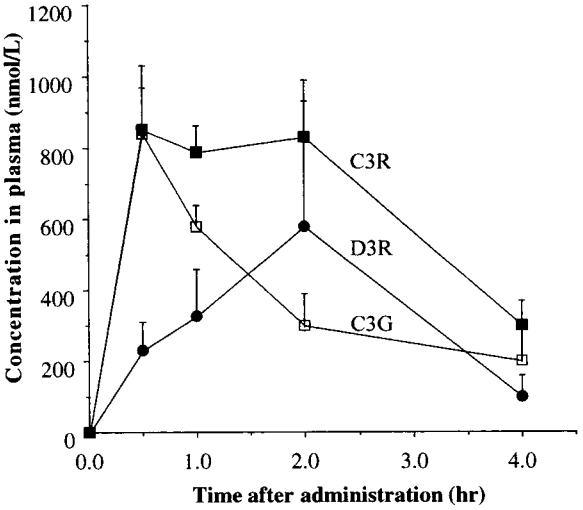

Figure 2. Time course of changes in concentrations of D3R, C3R, and C3G in rat plasma after oral administration of a single dose of purified anthocyanin: D3R (●); C3R (■); C3G (□). Values are the mean ± SD for three rats.

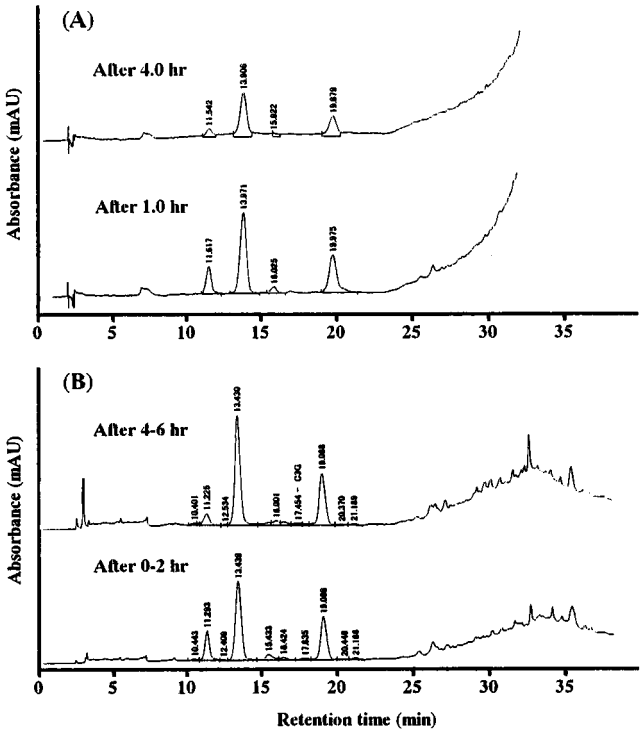

Figure 3. Typical HPLC profiles for subject 7 after oral ingestion of BCA: (A) HPLC chromatogram obtained in analysis of plasma 1.0 and 4.0 h after intake of BCA; (B) HPLC chromatogram obtained in analysis of urine 0−2 and 4−6 h after intake of BCA.

Human Study. It was confirmed that the human plasma contained no detectable anthocyanins before the intake of BCA. After oral ingestion of BCA concentrate [1.98−2.38 g/man, 33 mg/kg of body weight, corresponding to 6.24 μmol (3.58 mg) as BCA, 2.75 μmol (1.68 mg) as D3R, 2.08 μmol (1.24 mg) as C3R, 1.04 μmol (0.488 mg) as D3G, and 0.37 μmol (0.165 mg) as C3G/kg of body weight], the plasma and urinary concentrations of the four components were measured for 8 h. Figure 3 shows typical HPLC profiles of plasma (A) 1.0 and 4.0 h after intake of BCA and of urine (B) obtained in the periods 0−2 and 4−6 h after intake in the case of subject 7. D3R,

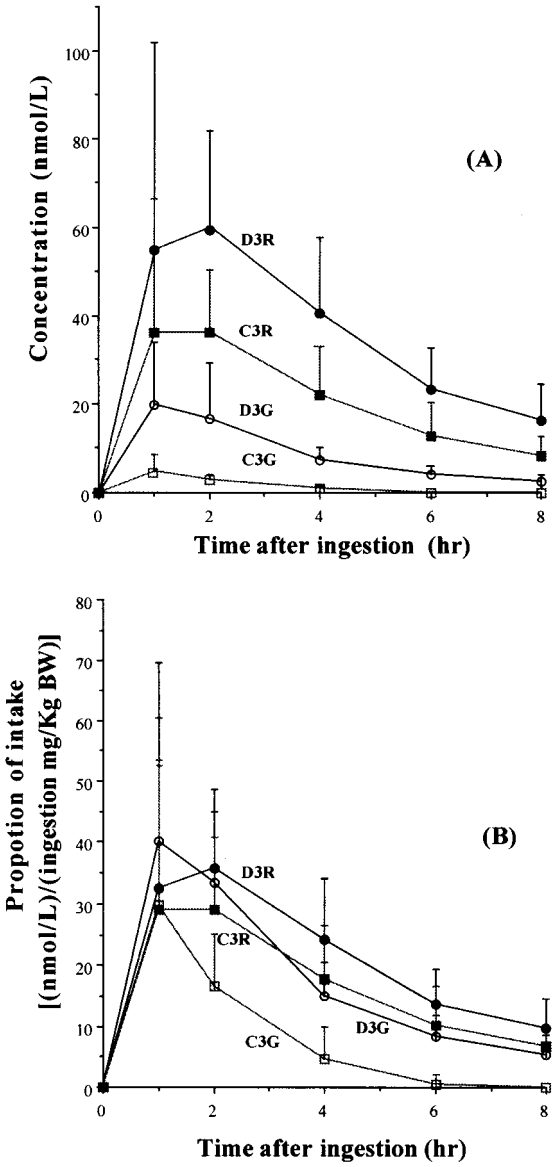

Figure 4. Time course of changes in concentrations of D3R, C3R, D3G, and C3G in human plasma after oral intake of a single dose of BCA: (A) concentration of anthocyanins in human plasma (nmol/L); (B) proportion of anthocyanins adsorbed and detected in human plasma [(nmol/L)/(mg ingested/kg of body weight)]; D3R (●); C3R (■); D3G (○); C3G, (□). Values are the mean ± SD for eight subjects.

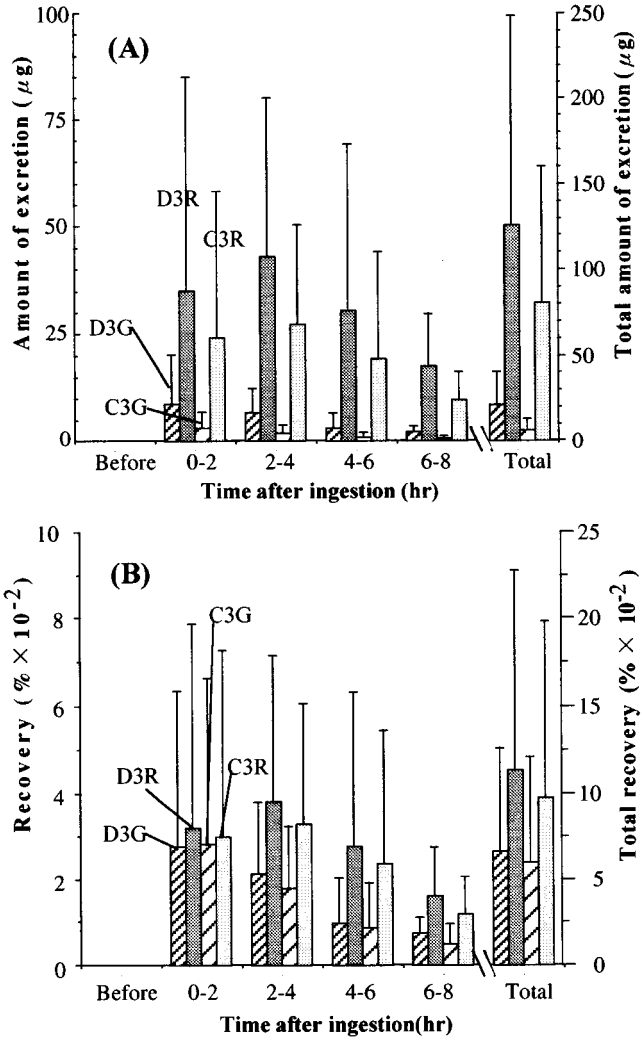

Figure 5. Time course of changes in recovery of D3R, C3R, D3G, and C3G excreted in human urine after oral intake of a single dose of BCA: (A) amount of anthocyanins excreted into urine (μg); (B) recovery of anthocyanins excreted into urine (% × 10^{-2}); bars represent, from left to right in each grouping, D3G, D3R, C3G, and C3R. Values are the mean ± SD for eight subjects.

C3R, D3G, and C3G were detected in both the plasma and the urine as the intact form.

Figure 4 shows the time course of changes in the concentrations of D3R, C3R, D3G, and C3G after ingestion of the BCA concentrate. The concentration reached maxima of 59.9 ± 21.6 nmol/L at 2.0 h postintake in the case of D3R, 36.1 ± 14.3 nmol/L at 2.0 h postintake in the case of C3R, 19.5 ± 14.4 nmol/L at 1.0 h postintake in the case of D3G, and 4.9 ± 3.8 nmol/L at 1.0 h postintake in the case of C3G and then gradually decreased. Figure 4B shows the change in the relative proportion of each anthocyanin in plasma [(nmol/L)/(mg ingested/kg of body weight)] after intake of the BCA concentrate. The decrease in the levels of the rutinosides (D3R and C3R) was gentler than that in the case of the glucosides (D3G and C3G).

Figure 5A shows the time course of changes in urinary excretion of the four components, as determined by HPLC. The anthocyanin (nanomoles)/creatinine (grams) ratio showed a similar profile. The urinary excretion reached maxima of 70.5 ± 60.5 nmol (43.1 ± 37.0 μg) between 2 and 4.0 h in the case of D3R, 45.8 ± 38.7 nmol (27.3 ± 23.1 μg) between 2 and 4.0 h in the case of C3R, 19.0 ± 24.1 nmol (8.88 ± 11.3μg) between 0 and 2.0 h in the case of D3G, and 6.74 ± 8.92 nmol (30.3 ± 4.02 μg) between 0 and 2.0 h in the case of C3G and then gradually decreased. Figure 5B shows the recovery of D3R, C3R, D3G, and C3G excreted in urine as a percentage of the dose ingested. Comparison of the four components shows that the excretion levels were almost the same (0.03%) at 0−2.0 h postintake, but thereafter the behavior of the rutinosides (D3R and C3R) differed from that of the glucosides (D3G and C3G). In the case of the latter, the excretion level began to decrease after 0−2 h postintake, whereas in the case of the former there was an increase in the excretion level, reaching a maximum at 2.0−4.0 h postintake. The total urinary recoveries of D3R, C3R, D3G, and C3G were calculated to be 0.11 ± 0.11, 0.098 ± 0.10, 0.066 ± 0.060, and 0.060 ± 0.061% of the dose ingested, respectively, for the 8-h period after intake.

Table 1. Pharmacokinetic Parameters of D3R, C3R, D3G, and C3G in Rats and Humans after a Single Oral Administration[a]

expt in	component	t_{max} (h)	C_{max} (nmol/L)	$t_{1/2}$ (h)	AUC_{0-obs} (nmol·h/L)
rats ($n = 3$)	D3R	2.0	580 ± 410	0.79	1330
	C3R	0.5	850 ± 120	1.36	2540
	C3G	0.5	840 ± 190	2.08	1510
humans ($n = 8$)	D3R	1.75 ± 1.04	73.4 ± 35.0	3.18 ± 1.33	287.9 ± 110.1
	C3R	1.50 ± 0.53	46.3 ± 22.5	3.45 ± 2.74	167.6 ± 74.9
	D3G	1.50 ± 0.53	22.7 ± 12.4	4.19 ± 4.30	68.8 ± 27.4
	C3G	1.25 ± 0.46	5.0 ± 3.7	1.34 ± 0.42	9.1 ± 7.1

[a] Values are the mean ± SD.

Pharmacokinetic Parameters. The data on plasma concentrations of BCA components in the case of both rats and humans were analyzed using a noncompartment model, and the pharmacokinetic parameter values obtained are summarized in Table 1. In the rat study, in which each component was administered at the same dosage (800 μmol/kg of body weight), the t_{max} values of D3R, C3R, and C3G were found to be 2.0, 0.5, and 0.5 h, respectively, and the C_{max} values were 580 ± 410, 850 ± 120, and 840 ± 190 nmol/L, respectively. The total area under the concentration time curve (AUC_{0-4h}) varied in the order C3R > C3G ≥ D3R, and the value in the case of C3R was 1.7−1.9-fold higher. In the human study involving the intake of a mixture of the four components, the t_{max} values varied in the order D3R > C3R = D3G > C3G. The C_{max} values varied in the order D3R > C3R > D3G > C3G, which was the same order as that of the amounts of the four components in the mixture ingested, and the AUC_{0-8h} values varied in the same order.

DISCUSSION

Recently, much attention has been paid to the biological functions of dietary flavonoids. Indeed, anthocyanins, natural pigments of the flavonoid family, have been shown to have multiple biological effects (*3*, *11*, *15*). In view of their effects, the bioavailability is considered to be an important issue.

In the present study, three purified anthocyanins, D3R, C3R, and C3G, were orally administered to rats at the same dosage (800 μmol/kg of body weight), and these were found to be absorbed and distributed to the blood as the intact forms. Comparison of the time course of changes in plasma concentrations of the three components showed the following features: (1) D3R and C3R were directly absorbed and distributed to the blood, and the plasma concentrations increased in the period up to 2.0 h postadministration and then gradually decreased. (2) In the case of C3G the C_{max} was reached at 0.5 h postadministration, and then the plasma concentration decreased, showing a profile similar to that reported by Tsuda et al. (*7*). (3) The decrease in the levels of the rutinosides was gentler than that in the case of the glucosides. The pharmacokinetic parameter values also supported these features, as the AUC_{0-4h} values varied in the order C3R > C3G ≥ D3R. In the human study, in which the subjects ingested BCA concentrate, the plasma concentrations of individual components were found to be consistent with those observed in the case of rats, as shown in Figure 4B. Both D3R and C3R showed high levels (59.9 ± 21.6 and 36.1 ± 14.3 nmol/L) until 2 h postintake and then gradually decreased, whereas the levels of D3G and C3G reached maxima (19.5 ± 14.4 and 4.9 ± 3.8 nmol/L) at 1 h postintake and then decreased more rapidly. In terms of the pharmacokinetics, the C_{max} and AUC_{0-8h} values varied in the order D3R > C3R > D3G > C3G, the same order as that of the amounts of the four components in the mixture ingested.

Our results demonstrate that D3R and C3R are directly absorbed and distributed to the blood and excreted in urine as the intact forms. There have been only two reports of studies on absorption of flavonoid rutinosides in humans, concerning rutin (quercetin 3-*O*-β-rutinoside) (*5*) and naringin (naringenin 7-*O*-β-rutinoside) (*12*). The absorption of rutin was markedly delayed as seen 6 h after intake, whereas naringin was rapidly excreted into urine within 2 h after intake. Our present study indicates that absorption and excretion of D3R and C3R occur in a manner similar to that in the case of naringin. Furthermore, comparison of the anthocyanin 3-rutinosides and the glucosides in terms of the time course of changes in plasma and urinary concentrations revealed a difference in t_{max} values and in the rate of decline after the maximum had been reached. This suggests the possibility that the absorption and excretion were influenced by the difference in structural features of the glucose moiety. Rapid absorption of BCA and naringenin 7-rutinoside is considered to occur through the hexose transport pathway, because the feature is consistent with those of quercetin glucosides reported in the following two papers. Hollman et al. (*16*) proposed that quercetin glucosides were rapidly absorbed within 30 min of ingestion through sodium-dependent glucose transporter 1. Gee et al. (*17*) reported that intestinal transport of the glucosides involved both deglycosylation and interaction with the hexose transport pathway, which could transport intact glucosides more quickly than the deglycosylated aglycons.

After oral ingestion of BCA (6.24 μmol/kg of body weight), the concentrations of the total BCA in human plasma reached 115 nmol/L as shown in Figure 4A, and the feature is considered to be reflected in the urinary excretion profile of Figure 5A. These profiles indicate that anthocyanin 3-glycosides can be absorbed rapidly within 2 h of ingestion and are excreted in urine as the intact forms. Judging from the plasma concentration and urinary recovery, the absorption rate of BCA as proportion of intake is supposed to be very low (<1%). Miyazawa et al. (*8*) also reported a similar level increasing that C3G in human human plasma reached 29 ± 10 nmol/L after a similar dosage of C3R (6.01 μmol/kg of body weight), but there has been no authorized information about the low rate. Some of the glucosides have possibilities to be deconjugated by intestinal hydrolase, but the rutinosides are supposed to remain without hydrolysis (*17*, *18*). Further absorption studies are needed to resolve this discrepacny.

It is noteworthy that oral intake of the same BCA concentrate at one-fourth the dosage by healthy human

subjects has been shown to result in improvement of dark adaptation and transient alterations in vision induced by work at VDT (*11*). These plasma concentrations were a little lower than those at which several anthocyanins were reported to show biological activity in in vitro studies, such as oxygen radical scavenging activity (IC_{50} of D3G = 1600 ± 100 nmol/L) (*19*) and inhibition of cGMP-phosphodiesterase (IC_{50} of malvidin 3-glucoside = 5600 ± 1400 nmol/L) (*3*). Our analysis in this study was limited to four components as intact glycosides, and the level of urinary excretion in humans was low, only 0.11 ± 0.05% (w/w) of the dose ingested. To obtain a higher proportion of recovery, it will be necessary to perform further studies tracing both the intact compounds and their metabolites in blood and the digestive tract.

The present study demonstrates that orally administered D3R and C3R are directly absorbed and distributed to the blood and excreted into urine in rats and humans. This is the first study to provide evidence of direct absorption and excretion of anthocyanin 3-*O*-*β*-rutinosides as the intact forms.

ABBREVIATIONS USED

AUC, total area under the concentration−time curve; BCA, black currant anthocyanins; C3G, cyanidin 3-*O*-*β*-glucoside; C3R, cyanidin 3-*O*-*β*-rutinoside; D3G, delphinidin 3-*O*-*β*-glucoside; D3R, delphinidin 3-*O*-*β*-rutinoside; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid.

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