

Identification and Quantification of Metabolites of Orally Administered Naringenin Chalcone in Rats

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Naringenin chalcone is the main active component of tomato skin extract, which has an antiallergic activity. In this study, naringenin chalcone was orally administered to rats, and the chemical structures and levels of the major metabolites in the plasma and urine of rats were determined. HPLC analysis indicated the presence of three major metabolites in the urine. LC-MS and NMR analyses tentatively identified these as naringenin chalcone-2'-*O*- β -D-glucuronide, naringenin-7-*O*- β -D-glucuronide, and naringenin-4'-*O*- β -D-glucuronide. Naringenin chalcone-2'-*O*- β -D-glucuronide was the only metabolite detected in the plasma, and its peak plasma level was observed 1 h after naringenin chalcone administration. Naringenin chalcone-2'-*O*- β -D-glucuronide also inhibited histamine release from rat peritoneal mast cells stimulated with compound **48/80**. This activity might contribute to the antiallergic activity of naringenin chalcone in vivo. To the best of the authors' knowledge, this study is the first to report determination of naringenin chalcone metabolites in rat plasma and urine.

KEYWORDS: Naringenin chalcone; metabolite; naringenin chalcone glucuronide; antiallergy

INTRODUCTION

Flavonoids are polyphenolic compounds that are present in foods and beverages of plant origin. Epidemiological studies have shown that the dietary intake of flavonoids is associated with biological effects such as a lower incidence of cardiovascular disease and cancer (1, 2). Naringenin is a bioactive flavonoid, and the inhibition effect of naringenin against hepatitis C virus was reported recently (3). It is very important to evaluate the bioavailability of flavonoids to determine whether the absorbed flavonoids have biological activity in vivo. There are some reports on the pharmacokinetics of flavonoids, such as naringenin, hesperetin, and quercetin (4–8).

We have recently reported that a tomato skin extract, which was prepared by extracting tomato skin with 60% (v/v) ethanol, inhibited histamine release from rat peritoneal mast cells stimulated with compound **48/80** and type I allergic reaction in mouse model (9). Furthermore, in our previous experimental study, we have confirmed that the main active component of tomato skin extract is naringenin chalcone (Figure 1) (9). We also reported that tomato skin extract could relieve the symptoms of perennial allergic rhinitis (10).

There are many reports on the bioavailability of naringenin (5–7), which is the cyclized structure of naringenin chalcone, but none on that of naringenin chalcone itself. In this study, to estimate the metabolism of naringenin chalcone, we examined the levels of its metabolites in plasma and urine of rats by using HPLC, LC-MS, and NMR analyses.

MATERIALS AND METHODS

Chemicals and Reagents. Naringenin was purchased from Funakoshi Co. (Tokyo, Japan). Compound **48/80** was purchased from Sigma Chemical Co. (St. Louis, MO). Uridine-5'-diphosphoglucuronic acid was purchased from Nacalai Tesque Inc. (Kyoto, Japan). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from Dojindo (Kumamoto, Japan). Rat liver microsomal protein (S-9) was obtained from Kikkoman Co. (Chiba, Japan). Other chemicals and solvents were purchased from Wako Pure Chemicals Co. (Osaka, Japan) and used without further purification.

Animals. Male Sprague–Dawley rats (250–300 g) were purchased from Japan SLC Co. (Shizuoka, Japan). These animals were acclimated in an environmentally controlled room (temperature, 23 ± 1 °C; humidity, $55 \pm 5\%$; and illumination time, 7:00 a.m. to 7:00 p.m.) and were raised on a type of MF standard diet (Oriental Yeast Co., Tsukuba, Japan) and water ad libitum for 1 week before the experiment. The experimental protocols were approved by the animal research control committee of Kikkoman Corp. and performed according to the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals. The researchers who used the animals receive annual instruction by the animal research control committee of Kikkoman Corp.

Preparation of Naringenin Chalcone from Tomato Skin Extract. Tomato (*Lycopersicon esculentum* Miller) skin extract containing polyphenols was prepared by incubating a mixture of the skin with 60% (v/v) ethanol at 60 °C for 2 h, followed by lyophilization. Preparation of naringenin chalcone was based on the identification method of naringenin chalcone, which was reported (9). Naringenin chalcone was separated from the tomato skin extract by HPLC. The column used was a 250 mm \times 15 mm i.d. Capcell Pak C18 column (Shiseido, Tokyo, Japan). Elution was carried out with acetonitrile/water (33:67, v/v) containing 0.1% trifluoroacetic acid at a flow rate of 5 mL/min at room temperature and monitored at 350 nm. The peak fraction was collected, concentrated under reduced pressure to remove the organic solvents, and then freeze-dried.

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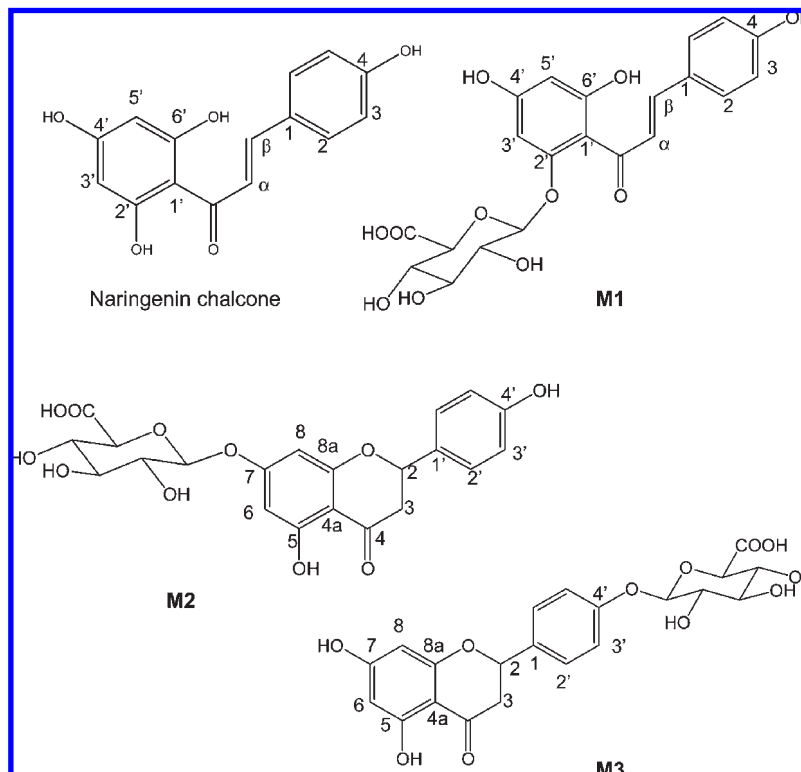


Figure 1. Chemical structures of naringenin chalcone and its metabolites.

Table 1. HPLC-MS Data for Metabolites of Naringenin

| retention time (min) | UV max (nm) | mass ion (ESI) | |
|----------------------|-------------|----------------------|-----------------|
| | | [M + H] ⁺ | major fragments |
| M1 | 16.9 | 365 | 449 273 |
| M2 | 10.4 | 283 | 449 273 |
| M3 | 11.4 | 283 | 449 273 |

The fraction was identified as naringenin chalcone by LC-MS (98% purity).

In Vivo Study. The rats were orally administered 0.5% carboxymethyl cellulose sodium salt solution (5 mL/kg of body weight) with or without naringenin chalcone (20 mg/kg). They were anesthetized with diethyl ether at 0, 0.5, 1, 2, 4, and 24 h after sample administration. Blood samples from the jugular vein were collected into heparinized tubes. They were centrifuged at 2000g for 5 min at 4 °C, and the supernatants were collected. Urine samples were collected hourly from the 0–10 and 10–24 h periods in test tubes. Each plasma and urine sample was mixed with an equal volume of methanol and centrifuged at 2500g for 10 min at 4 °C. The supernatant of each sample was collected in a test tube and also stored at –20 °C until further analysis.

Isolation of Metabolites from Plasma and Urine. Each sample was filtered through a cellulose acetate DISMIC filter (Advantec, Dublin, CA) and analyzed by HPLC. The column used was a 150 × 4.6 mm i.d. Capcell Pak C18-UG120 (Shiseido). Elution was carried out with acetonitrile/water (20:80, v/v) containing 0.1% trifluoroacetic acid at a flow rate of 1 mL/min at room temperature and monitored between 250 and 400 nm. Retention times of major metabolites, M1, M2, and M3, are given in Table 1. The limit of metabolite detection was 0.3 μM.

Enzymatic Synthesis of Related Glucuronides. We synthesized the corresponding glucuronides of naringenin chalcone enzymatically. Naringenin chalcone was dissolved in 50% (v/v) ethanol (20 mM, 2.5 mL) and mixed with 22 mL of a reaction buffer solution [0.5 mM HEPES (pH 5.0), 3 mM uridine-5'-diphosphoglucuronic acid, 0.1 mM MgCl₂, and 25 mL of rat liver microsomal protein solution]. The mixture was incubated at 25 °C. After 3 h, it was mixed with an equal volume of methanol and centrifuged at 2000g for 10 min at 4 °C. The supernatant was concentrated under vacuum, dissolved in dimethyl sulfoxide, filtered through a DISMIC filter

(Advantec), and injected into the HPLC system. The column used was a 250 × 10 mm i.d. YMC-Pack Pro C18 column (YMC, Kyoto, Japan). Elution was carried out with acetonitrile/water (25:75, v/v) containing 0.1% trifluoroacetic acid at a flow rate of 5 mL/min at room temperature and monitored between 250 and 400 nm. Three major fractions were collected, concentrated under reduced pressure to remove the solvents, and then freeze-dried. They were analyzed by LC-MS and NMR.

HPLC System. HPLC analysis used a JASCO HPLC system consisting of a pump PU-2080 Plus, an autoinjector AS-2057 Plus, and a multiwavelength detector MD-2010 Plus (JASCO, Tokyo, Japan).

LC-MS Analysis. LC-MS analysis used a system of JMS-BU30 (JEOL, Tokyo, Japan). The column used was a 150 × 4.6 mm i.d. Capcell Pak C18-UG120 (Shiseido) for calculating the molecular weight. The LC-MS conditions were as follows: atmospheric pressure chemical ionization (APCI) in the positive ion mode; desolving plate, 220 °C; orifice 1, 70 °C; and lens ring voltage, 50 V. Elution was carried out with acetonitrile/water (20:80, v/v) containing 0.1% acetic acid at a flow rate of 1 mL/min at room temperature. MS was performed in the selected ion monitoring mode (SIM).

NMR Analysis. To determine the structure of naringenin chalcone metabolites, one- and two-dimensional (1D- and 2D) NMR spectra, including ¹H–¹H correlation spectroscopy (COSY), ¹H–¹³C heteronuclear multiple quantum coherence (HMQC), and ¹H–¹³C heteronuclear multiple bond connectivity (HMBC), were recorded in CD₃OD at 300 K using a Bruker Avance 500 spectrometer (Bruker Biospin, Tsukuba, Japan), operated at 500.1 MHz for ¹H and at 125.7 MHz for ¹³C. A summary of ¹H and ¹³C resonance of isolated metabolites is given in Tables 2 and 3.

Assay of the Inhibition of Histamine Release from Rat Peritoneal Mast Cells. Preparation of the mast cells and assay of the inhibition of histamine release from rat peritoneal mast cells were based on reported methods (11). Rat peritoneal mast cells were collected from the peritoneal cavity after the injection of a buffer solution [0.15 M NaCl, 3.7 mM KCl, 3.0 mM Na₂HPO₄, 3.5 mM KH₂PO₄, 0.9 mM CaCl₂, 5.6 mM glucose, and 0.1% (w/v) gelatin] and then partially purified by centrifugation. Test samples, which were dissolved in the buffer solution containing 0.5% (v/v) dimethyl sulfoxide, were added to the cell suspension (2.5 × 10⁵ cells/mL in 80 μL) and subsequently incubated at 37 °C for 10 min. The cells were then

Table 2. ^1H and ^{13}C NMR Spectroscopic Data for Naringenin Chalcone and **M1** in CD_3OD

| position | naringenin chalcone | | M1 | |
|--------------|---------------------|---------------------|---------------------|---------------------|
| | δ_{H} | δ_{C} | δ_{H} | δ_{C} |
| chalcone C=O | | 194.2 | | 194.5 |
| α | 8.06 | 125.6 | 7.98 | 125.9 |
| β | 7.69 | 143.6 | 7.66 | 144.1 |
| 1 | | 128.5 | | 128.5 |
| 2, 6 | 7.48 | 131.3 | 7.62 | 131.8 |
| 3, 5 | 6.81 | 116.8 | 6.83 | 116.9 |
| 4 | | 161.0 | | 161.1 |
| 1' | | 105.9 | | 107.8 |
| 2' | | 166.0 | | 161.6 |
| 3' | 5.84 | 96.0 | 6.19 | 95.7 |
| 4' | | 166.2 | | 165.7 |
| 5' | 5.84 | 96.0 | 6.01 | 98.7 |
| 6' | | 166.0 | | 165.7 |
| 1'' | | | 5.22 | 102.0 |
| 2'' | | | 3.59–3.52 | 77.7–72.9 |
| 3'' | | | 3.59–3.52 | 77.7–72.9 |
| 4'' | | | 3.66 | 77.7–72.9 |
| 5'' | | | 4.02 | 77.7–72.9 |
| 6'' | | | | 171.9 |

Table 3. ^1H and ^{13}C NMR Spectroscopic Data for **M2** and **M3** in CD_3OD

| position | M2 | | M3 | |
|----------|---------------------|---------------------|---------------------|---------------------|
| | δ_{H} | δ_{C} | δ_{H} | δ_{C} |
| 2 | 5.39 | 80.7 | 5.40 | 80.1 |
| 3 | 3.17, 2.75 | 44.1 | 3.09, 2.73 | 44.1 |
| 4 | | 198.6 | | 197.5 |
| 4a | | 105.1 | | 103.4 |
| 5, 7, 8a | | 166.6–164.6 | | 168.4–164.7 |
| 6 | 6.18 | 97.9 | 5.89 | 97.1 |
| 8 | 6.18 | 96.9 | 5.89 | 96.2 |
| 1' | | 130.8 | | 134.6 |
| 2', 6' | 7.32 | 129.1 | 7.43 | 128.8 |
| 3', 5' | 6.81 | 116.3 | 7.12 | 118.0 |
| 4' | | 159.1 | | 159.0 |
| 1'' | 5.07 | 101.0 | 5.01 | 102.3 |
| 2'' | 3.48 | 74.3 | 3.50 | 74.6 |
| 3'' | 3.48 | 77.1 | 3.50 | 77.3 |
| 4'' | 3.59 | 72.8 | 3.62 | 73.0 |
| 5'' | 4.02 | 76.5 | 3.99 | 76.6 |
| 6'' | | 172.0 | | 172.1 |

stimulated with compound **48/80** (final concentration = 0.5 $\mu\text{g}/\text{mL}$) for 10 min. After centrifugation of the cell suspension at 1500g for 5 min at 4 °C, each supernatant was mixed with the same volume of HCl (0.1 N) to stop histamine release from the cells. The amount of histamine in each solution was analyzed by HPLC using the on-column derivatization method with *o*-phthalaldehyde (12).

Statistical Analysis. Each data value is expressed as the mean value and its standard error (SEM). Statistical analysis was performed by nonparametric Mann–Whitney's U test for comparisons between the groups administered vehicle or naringenin chalcone. All statistical analyses were two-tailed, and statistical significance was established at $p < 0.05$. Statistical analyses were performed using SPSS Systems (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Detection of Naringenin Chalcone Metabolites in Rat Urine. In the present study, we identified the three major metabolites of naringenin chalcone and determined their structures by LC-MS and NMR analyses. **Figure 2** shows representative chromatograms of rat urine samples 1 h after the administration of vehicle (**Figure 2A**) or naringenin chalcone (**Figure 2B**), respectively. **Table 1** shows the mass data of these metabolites. The positive APCI-MS of **M1** showed molecular ion peaks ($[\text{M} + \text{H}]^+$) at m/z 273 and 449 corresponding to a naringenin chalcone and a naringenin chalcone monoglucuronide, respectively. The UV absorption maximum of **M1** was the same as that of naringenin chalcone (**Figure 3A**). **M2** and **M3** showed the same $[\text{M} + \text{H}]^+$ peaks of **M1**, but their UV spectra were the same as that of naringenin (**Figure 3B,C**). **M2** and **M3** appear to be two different types of naringenin monoglucuronide, considering that their retention times were inconsistent.

Previous studies indicated that metabolites of naringenin were found in plasma and urine as glucuronides (13, 14). In our study, naringenin chalcone might be extensively metabolized by glucuronidases. The amount of the three isolated metabolites of naringenin chalcone in the urine sample was too little to be analyzed by NMR. We prepared the glucuronides of naringenin chalcone by incubating naringenin chalcone with rat liver microsomes and uridine-5'-diphosphoglucuronic acid. Three major peaks were detected from the reaction solution by HPLC (data not shown). These were purified and analyzed by HPLC and LC-MS. They were found to be identical to the same compounds of the three metabolites of naringenin chalcone in urine, determined from retention times, their spectra, and molecular weights (data not shown). These enzymatically synthesized metabolites were used for NMR analysis.

Identification of Metabolites of Naringenin Chalcone. For detailed structural analysis, **M1** was analyzed by NMR (**Table 2**). The ^1H NMR spectrum of naringenin chalcone glucuronide showed nonequivalence of the two aromatic protons (H-3' and H-5') and downfield shifts to δ_{H} 6.19 and 6.01, respectively, in comparison with those of naringenin chalcone at δ_{H} 5.84 (2H, br s, H-3' and H-5'). Comparison of the ^{13}C NMR spectrum of naringenin chalcone glucuronide with that of naringenin chalcone revealed that two equivalent signals of naringenin chalcone at δ_{C} 166.0 (C-2' and C-6') and δ_{C} 96.0 (C-3' and C-5') shifted to nonequivalent signals of naringenin chalcone glucuronide at δ_{C} 165.7 (C-6'), 161.6 (C-2'), 98.7 (C-5'), and 95.7 (C-3') as determined by HMQC and HMBC data. In addition, on comparison of the HMBC signals of naringenin chalcone glucuronide and naringenin chalcone, the upfield shifted carbon of naringenin chalcone glucuronide at δ_{C} 161.6 (C-2') correlated with the proton signal at δ_{H} 5.22 (H-1''). The glycoside linkage was assigned as β on the basis of the anomeric proton signal at δ_{H} 5.22 (d, $J = 7.3$ Hz, H-1''). These results indicate that the C-2' position of the naringenin chalcone skeleton is modified and bonded glycosidically with glucuronic acid in the β form (**Figure 1**). **M1** was tentatively identified as naringenin chalcone-2'-*O*- β -D-glucuronide.

In addition, **M2** and **M3** were identified as naringenin-7-*O*- β -D-glucuronide and naringenin-4'-*O*- β -D-glucuronide, respectively, by specific chemical shifts in the ^1H NMR spectra and correlations in the HMBC spectra (**Table 3**) (13). The proton signals in the conjugated aromatic ring were shifted downfield in comparison with those of naringenin. In **M2**, the proton signal at δ_{H} 6.18 (2H, m, H-6, 8) in the conjugated aromatic ring was equivalently shifted downfield in comparison with those of naringenin at δ_{H} 5.89 (2H, H-6, 8). In **M3**, the carbon signal at δ_{C} 159.0 (C-4') was

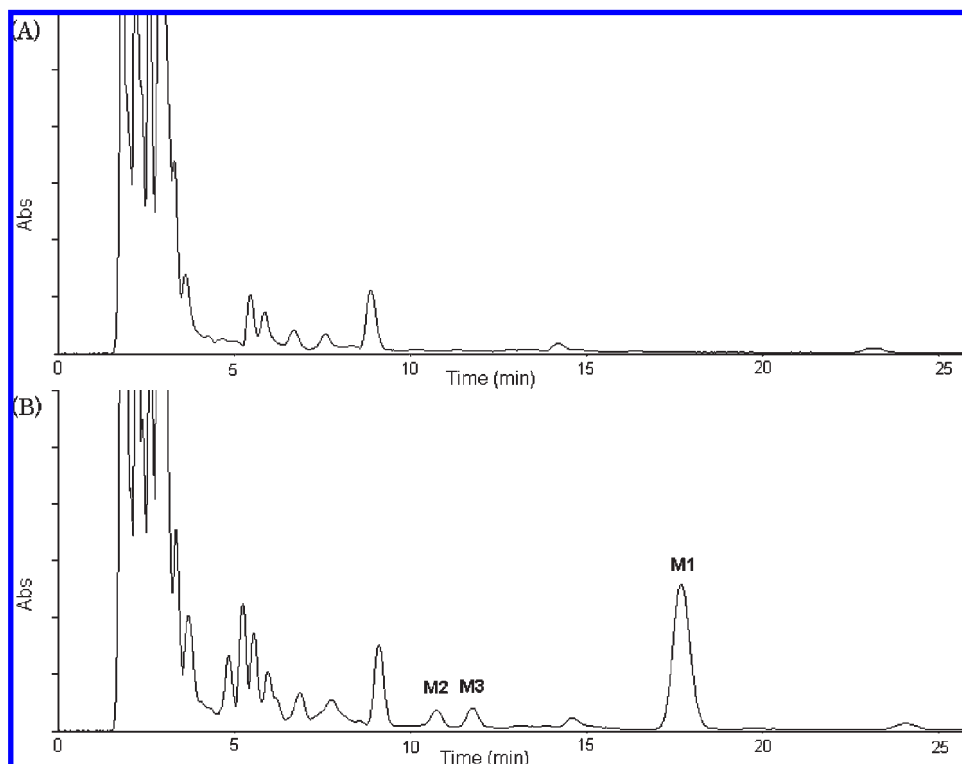


Figure 2. Representative HPLC chromatograms of naringenin chalcone metabolites in urine at 310 nm. The urine sample was collected 1 h after the administration of vehicle (A) or 20 mg/kg of naringenin chalcone (B).

observed in correlation with the proton signal at δ_{H} 5.01 (1H, H-1'') with HMBC analysis, as well as downfield shifts of the proton signals at δ_{H} 7.30 (2H, H-2',6') and δ_{H} 6.81 (2H, H-3',5') in naringenin to δ_{H} 7.43 (2H, H-2',6') and δ_{H} 7.12 (2H, H-3',5'). The anomeric proton of glucuronic acid ($J > 7.0$ Hz) exhibited HMBC correlations to an A-ring carbon (C-7) and a B-ring carbon (C-4'), indicating β positions of naringenin-7-*O*- β -D-glucuronide and naringenin-4'-*O*- β -D-glucuronide, respectively (Figure 1).

LC-MS and NMR analyses showed that the major metabolites in urine were naringenin chalcone-2'-*O*- β -D-glucuronide, naringenin-7-*O*- β -D-glucuronide, and naringenin-4'-*O*- β -D-glucuronide. This study reports for the first time the presence of naringenin chalcone-2'-*O*- β -D-glucuronide in rat plasma and urine after naringenin chalcone administration. It has been reported that phloretin (2',4',6',4-tetrahydroxydihydrochalcone), which has a chalcone form like naringenin chalcone, is metabolized to a phloretin glucuronide that has a chalcone form (15). Therefore, it is reasonable to assume that naringenin chalcone was metabolized to naringenin chalcone glucuronide, keeping a chalcone form. Naringenin chalcone is metabolized to naringenin by chalcone isomerase. Some chalcone isomerase has been isolated from human fecal bacteria (16). Several studies have noted that naringenin is metabolized to naringenin glucuronides (13, 14, 17). The glucuronosyltransferase, which is involved in the synthesis of glucuronide metabolites of flavonoids, has been found in the intestines and livers of rat and humans (18, 19). Therefore, some of the naringenin chalcone might be converted to naringenin by chalcone isomerase of fecal bacteria and metabolized to naringenin glucuronides.

Time Course Analysis of Naringenin Chalcone Metabolites in Plasma and Urine. The time-course characteristics for changes in the concentrations of M1, M2, and M3 in the plasma and urine after naringenin chalcone administration are shown in Figures 4 and 5. The enzymatically synthesized glucuronides were used as

quantitative standards. When naringenin chalcone was orally administered to rats, only M1 was detected in plasma (Figure 6). The peak was not detected in the plasma of rats administered vehicle. Two types of naringenin glucuronide were not detected in the plasma samples. The peak plasma level for M1 was observed 1 h after naringenin chalcone administration, and its concentration was $5.0 \pm 1.0 \mu\text{M}$ (Figure 4). M1 had a half-life of 5.5 ± 1.7 h. The level of M1 in the plasma decreased to nearly undetectable levels at 24 h after administration. It had been reported that the glucuronidation of naringenin occurs in the intestinal epithelium (20). Furthermore, naringenin chalcone might be glucuronized in the intestinal epithelium and transferred to the plasma shortly after oral administration. The absence of naringenin glucuronides in the plasma might indicate that once these have been released into the bloodstream, they are rapidly removed by excretion via the kidney. Such pharmacokinetics had been reported for quercetin metabolites (8). Further investigation is required to determine the pathway through which naringenin glucuronides are synthesized from naringenin chalcone in vivo.

Figure 5 shows the cumulative excretion of the three metabolites of naringenin chalcone in the urine. The three metabolites were observed 1 h after oral administration. These peaks were not detected in the plasma of rat administered vehicle. M1 was excreted more rapidly than two types of naringenin glucuronide in the urine. The level of M1 reached a peak between 2 and 4 h and increased throughout a 24 h period, whereas those of two types of naringenin glucuronide, M2 and M3, reached a peak between 3 and 6 h. The three metabolites were not detected 48 h after administration (data not shown). The recovery rates of M1, M2, and M3 in urine were 7.5 ± 0.4 , 5.4 ± 0.4 , and $7.9 \pm 0.1\%$ of intake, respectively. The total cumulative amount of the three metabolites after 24 h was $20.9 \pm 1.6\%$ of the dosage. Because the recovery of the metabolites in the urine was only 21%, a large amount of the ingested dose was unaccounted for. It has been reported that naringenin (21) and phloretin (22), naringenin

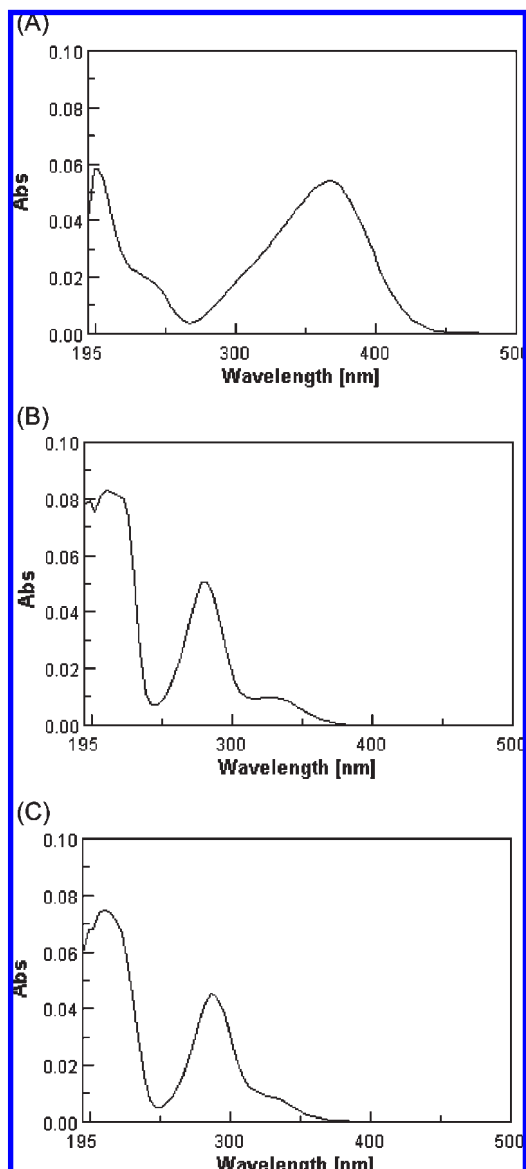


Figure 3. UV spectra of M1 (A), M2 (B), and M3 (C) in the urine of rat administered naringenin chalcone.

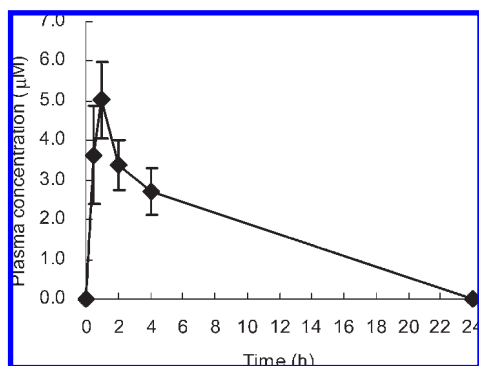


Figure 4. Plasma concentration of naringenin chalcone-2'-O-β-D-glucuronide in rats after oral administration of naringenin chalcone. Each value represents the mean ± SEM ($n = 7$).

dihydrochalcone, is metabolized to the corresponding glucuronide and ring-fission metabolites such as *p*-hydroxyphenylpropionic acid. There is some evidence to suggest that the enzyme in

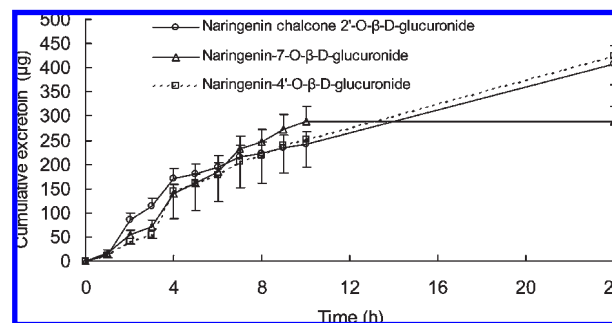


Figure 5. Cumulative urinary excretion curves of naringenin chalcone metabolites of rats after oral administration of naringenin chalcone. Each value represents the mean ± SEM ($n = 10$).

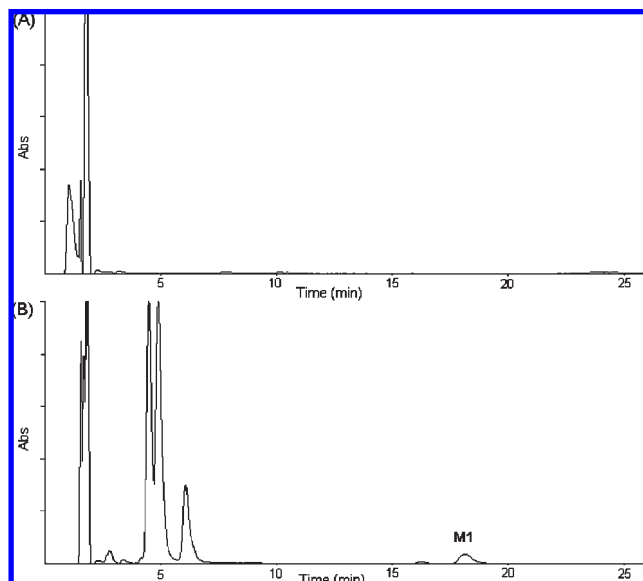


Figure 6. Representative HPLC chromatograms of naringenin chalcone metabolites in plasma at 310 nm. The plasma sample was collected 1 h after the administration of vehicle (A) or 20 mg/kg of naringenin chalcone (B).

liver microsomes (23, 24) and the intestinal microflora (25, 26) were capable of effecting flavonoid ring-fission and conversion of flavonoids to related phenolic acids and phloroglucinol. Naringenin chalcone might be converted to a phenolic acid that was not identified in this study. Further investigation is required to detect the ring-fission metabolites of naringenin chalcone.

Effect of Metabolites on Histamine Release from Rat Peritoneal Mast Cells. Recently, several studies have reported that flavonoid metabolites, such as (+)-catechin and quercetin metabolites, have biological activities (5, 27, 28). To determine the anti-allergic properties of the three metabolites of naringenin chalcone, we studied histamine release assay with rat peritoneal mast cells. All of the metabolites exhibited dose-dependent inhibition (data not shown). The IC_{50} values of M1, M2, and M3 were 510.3 µM, 3.2 mM, and 2.8 mM, respectively. M1 showed stronger anti-allergic activity than M2 and M3. The activity of M1 might be lower than that of naringenin chalcone ($IC_{50} = 33.1$ µM) in this study. Recently, it was reported that the metabolites of polyphenols can be deconjugated during inflammation and contribute to its aglycone bioactivity (29). M1 might be deconjugated and contribute to the anti-allergic activity of naringenin chalcone in vivo. Further investigation is required to detect the active form in the inflammatory site.

In conclusion, the major metabolites of naringenin chalcone were tentatively identified as naringenin chalcone-2'-O- β -D-glucuronide, naringenin-7-O- β -D-glucuronide, and naringenin-4'-O- β -D-glucuronide. These three metabolites were detected in the urine, but only naringenin chalcone-2'-O- β -D-glucuronide was detected in the plasma. Naringenin chalcone-2'-O- β -D-glucuronide inhibited histamine release, and this might contribute to the antiallergic activity of naringenin chalcone in vivo. The bioavailability of naringenin chalcone must be studied to confirm the mechanism of action.

ABBREVIATIONS USED

HEPES, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; NMR, nuclear magnetic resonance; APCI, atmospheric pressure chemical ionization; SIM, selected ion monitoring mode; COSY, ^1H - ^1H correlation spectroscopy; HMQC, ^1H - ^{13}C heteronuclear multiple quantum coherence; HMBC, ^1H - ^{13}C heteronuclear multiple bond connectivity; SEM, standard error of the mean.

ACKNOWLEDGMENT

We acknowledge the advice of Dr. Taichi Yamamoto, Children's Hospital Oakland Research Institute, for this study. We are grateful for the assistance of our laboratory staffs.

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