Eur J Nutr (2005) 44 : 1–9
DOI 10.1007/s00394-004-0482-2

ORIGINAL CONTRIBUTION

Seigo Baba
Naomi Osakabe
Midori Natsume
Akiko Yasuda
Yuko Muto
Kyoko Hiyoshi
Hirohisa Takano
Toshikazu Yoshikawa
Junji Terao

Absorption, metabolism, degradation and urinary excretion of rosmarinic acid after intake of *Perilla frutescens* extract in humans

Received: 27 May 2003
Accepted: 5 January 2004
Published online: 18 February 2004

S. Baba (✉) · N. Osakabe · M. Natsume · A. Yasuda · Y. Muto
Nutritional Science Center
Health and Bioscience Laboratories
Meiji Seika Kaisha, Ltd.
5-3-1, Chiyoda, Sakado-shi
Saitama 350-0289, Japan
Tel.: +81-492/84-7596
Fax: +81-492/84-7598
E-Mail: seigo_baba@meiji.co.jp

T. Hiyoshi
Graduate School of Comprehensive Human Science
University of Tsukuba
Ibaraki, Japan

H. Takano
Pathophysiology Research Team
National Institute for Environmental Studies
Ibaraki, Japan

T. Yoshikawa
1st Dept. of Medicine
Kyoto Prefectural University of Medicine
Kyoto, Japan

J. Terao
Dept. of Nutrition
School of Medicine
The University of Tokushima
Tokushima, Japan

Summary *Background* Rosmarinic acid (RA) is a natural polyphenolic substance contained in many Lamiaceae herbs such as *Perilla frutescens*. Previous studies have shown RA has antioxidative and anti-inflammatory activity. However, little is known on the absorption, metabolism, degradation and excretion of RA. *Aim of the study* The aim of this study in healthy humans was to determine the absorption, metabolism, and urinary excretion of RA after a single intake of perilla extract (PE). *Method* Six healthy men (mean age 37.2 ± 6.2 y and mean body mass index 22.0 ± 1.9 kg/m^2) were enrolled in the study that was a cross-over design involving single intakes of PE containing 200 mg RA and placebo with a 10 day interval between treatments. Blood samples were collected before intake and at designated time intervals, while urine samples were collected over the periods 0–6 h, 6–24 h and 24–48 h after intake. RA and its related metabolites in plasma and urine were measured by LC-MS. *Results* RA, methylated RA (methyl-RA), caffeic acid (CAA), ferulic acid (FA) and a trace of *m*-coumaric acid (COA) were detected in the urine after intake of PE. In plasma, RA, methyl-RA and FA were detected, with maximum levels obtained 0.5, 2 and 0.5 h after intake of PE, respectively. The majority of these components in both plasma and urine were present as conjugated forms (glucuronide and/or sulfated). The proportion of RA and its related metabolites excreted in the urine was 6.3 ± 2.2 % of the total dose, with approximately 75 % of these components being excreted within 6 h after intake of PE. *Conclusions* RA contained in PE was absorbed, conjugated and methylated following intake, with a small proportion of RA being degraded into various components, such as conjugated forms of CAA, FA and COA. These metabolites were then rapidly excreted in the urine.

Key words rosmarinic acid – *Perilla frutescens* – absorption – metabolism – human

Introduction

Rosmarinic acid (RA) is a natural polyphenolic component contained in various culinary herbs such as perilla (*Perilla frutescens L.*) [1], rosemary (*Rosmarinus officinalis L.*) [2], sage (*Salvia officinalis L.*) [3], mint (*Mentha arvense L.*) [4], basil (*Ocimum basilicum L.*) [5] and thyme (*Thymus vulgaris L.*) [6]. Epidemiological studies have shown there is a negative correlation between polyphenol consumption and the incidence of coronary heart disease and cancer [7, 8]. RA has been reported to

have *in vitro* antioxidative activity, such as the ability to scavenge superoxide and hydroxyl radicals [9], inhibit oxidation of low-density lipoprotein [10], and prevent formation of hydroperoxides in oil [11]. In addition, RA has been demonstrated to have several other actions including a potent anti-inflammatory activity resulting from decreased arachidonate formation [12], inhibition of hemolysis [13], and suppression of hyaluronidase and β-hexosaminidase activity [14]. An *in vivo* study carried out by Osakabe et al. found that perilla extract (PE) also reduced liver injury induced by lipopolysaccharide in D-galactosamine-sensitized mice and showed the main component responsible for this effect was RA contained in the extract [15]. Sanbongi et al. showed that RA also inhibited diesel exhaust particle-induced lung injury in mice, and attributed this action to reduced expression of pro-inflammatory molecules and enhancement of antioxidative activity [16].

Studies in rats have shown orally administered RA is present as intact and degraded and/or conjugated forms such as *m*-hydroxyphenylpropionic acid, *m*-coumaric acid (COA), and sulfated forms of caffeic acid (CAA), COA and ferulic acid (FA) that are subsequently excreted in the urine [17]. Topical administration of RA to the skin of rats resulted in the compound being absorbed percutaneously and distributed in blood, skin, bone and muscle while intravenous administration in these animals led to RA being detectable in various tissues such as lung, spleen, heart and liver [18]. These results show that, in rat, RA may be absorbed from the digestive tract and skin, and that following absorption RA is distributed in various tissues. In contrast, Nakazawa et al. demonstrated intake of PE in humans resulted in 1-*O*-(2,4,5-trimethoxycinnamoyl)-β-glucuronic acid being excreted in the urine, and proposed that this metabolite was derived from RA contained in the PE [19]. This finding indicates there are differences in the metabolism of RA between human and rat. As there is only limited detailed information on the absorption, metabolism and urinary excretion of RA in human we undertook an investigation of these factors in healthy male volunteers.

Methods

Subjects

Six healthy, male subjects volunteered to take part in the study that was approved by and performed under the guidelines of the Human Committee of the Tokushima University Hospital. Prior to the study the purpose of the investigations was explained to all subjects and informed consent obtained. Mean (± SD) age, body weight, and body mass index of the subjects were 37.2 ± 6.2 y (range: 31–50 y), 64.3 ± 6.6 kg (range: 54–72 kg), and 22.0 ± 1.9 kg/m^2 (range: 19–24 kg/m^2), respectively.

Chemicals

RA and COA was purchased from Extrasynthese (Genay, France). CAA, FA, cathecol-*O*-methyltransferase (COMT), S-adenosyl-L-methionine, d-saccharic acid 1.4-lactone, β-glucuronidase type VII-A and sulfatase type VIII [and type H-5 were purchased from Sigma Chemical Co. (St. Louis, MO). Other chemicals used in the study were of analytical or HPLC grade.

Synthesis and isolation of methylated RA as a standard

Synthesis of methylated RA (methyl-RA) was carried out according to the method of Spencer et al. [20]. Briefly, 4000 units COMT was dissolved in 200 ml phosphate buffer containing 20 mmol cysteine and 2 mmol magnesium chloride, pre-incubated at 37 °C for 10 min, followed by the addition of 500 ml of 7.1 mmol/l S-adenosyl-l-methionine and 100 ml of 1.0 mmol/l RA. This solution was then incubated for 24 h at 37 °C with nitrogen gas being bubbled continuously through the system. A further 200 ml of 7.1 mmol S-adenosyl-l-methionine was added 2 and 4 h after the start of incubation. The synthetic reaction was terminated by the addition of methanol, followed by dehydration of the solution *in vacuo* and re-suspension in 20 ml of 25 % methanol. This solution was then applied to a Discovery DSC-18 column (20 ml tubes 10 g Supelcosil; Supelco, Bellefonte, PA, USA), which was prepared with 100 ml methanol followed by 100 ml of water. The solid phase extract was eluted with H_2O/methanol in a stepwise fashion. The composition of each fraction was analyzed using liquid chromatography mass spectrometry (LC-MS), with the fraction detected at m/z 373 in the analysis being collected and concentrated *in vacuo*. This fraction corresponded to the mass number of monomethylated RA. A 1 µl aliquot of solution was injected into a reversed-phase semi-preparative HPLC column (Deverosil ODS-HG-5, 5 µm, 250 × 20 mm, Nomura Chemical Co., Aichi, Japan). The column was then eluted at room temperature for 30 min at a flow rate of 15 ml/min using a linear gradient of solvent, starting from 10 % methanol containing 0.05 % acetic acid to 45 % methanol containing 0.05 % acetic acid. The eluted compounds were monitored at a wavelength of 220 nm with peak fractions being collected, dehydrated, re-dissolved in 25 % methanol and then used for LC-MS analyses.

Preparation of perilla extract

The PE was prepared by Meiji Seika Kaisha Ltd. Fifty kilogram of fresh perilla leaves were extracted with 100 kg of 1 % w/v citric acid for 30 minutes at 90 °C. The extract was filtered, followed by the addition of 1.196 kg of dextrin, and then freeze-dried. The quantity of extract recovered was 3.196 kg. The concentration of polyphenolic substances, including RA, in this extract was determined by HPLC using a Deverosil HG-5 column (Nomura Chemical Co. Ltd., Aichi, Japan) using solvents A (0.1 % v/v TFA in distilled water) and B (0.1 % TFA v/v in acetonitrile) under the following conditions: 10–50 % linear gradient of A in B, flow rate 0.8 ml/min, and detection at 280 nm. The concentration of RA was 20 % w/w, while the concentration of dextrin was 36.7 %. The concentrations of CAA, FA and COA were all less than the detection limit of the method.

The test sample was supplied in tablet form and was composed of PE, lactose and cellulose powder. The placebo was prepared in a similar manner but without PE, with flavor and pigment being added in order to match the PE tablets because the PE was red powder and had a characteristic scent. These samples were stored shielded from the light at 20 ± 1 °C under nitrogen gas before being taken by the study subjects.

Study design

This study was carried out using a cross-over design with a 10 d wash-out period between PE and placebo administration. The same food and drinks were served to all subjects the day before PE or placebo intake until 24 h after intake. Baseline blood samples were drawn from the intermediate cubital vein after the subjects had fasted for 12 hours, followed by further samples being collected 0.5, 1, 2, 3, 6, 24 and 48 h after intake of PE or placebo. Plasma was separated by centrifugation at 1400 × g for 10 min at 4 °C, and stored at –80 °C under nitrogen gas until analysis. Urine samples were collected over the periods 0–6 h, 6–24 h and 24–48 h post-intake with the samples being frozen immediately at –80 °C with nitrogen gas until analysis. In order to avoid the effect of polyphenols derived from other food, for the period of the study the subjects were requested to consume foods containing minimal amounts of polyphenols, such as rice and raw fish. They were also requested to drink only water until 48 h after PE or placebo ingestion.

Measurement of RA, methyl-RA, CAA, FA, COA and their related metabolites in plasma and urine by HPLC

RA, methyl-RA, CAA, FA, COA and their conjugated forms (glucuronide and/or sulfate) were measured by treatment with either β-glucuronidase type VII-A for glucuronide hydrolysis, sulfatase type VIII for sulfate hydrolysis, or sulfatase type H-5 for combined glucuronide and sulfate hydrolysis according to previous reports [21, 22]. The amount of conjugated RA, methyl-RA, CAA, FA and COA in plasma samples was calculated as the amount after enzymatic hydrolysis minus the amount prior to hydrolysis for each compound. The frozen urine samples were thawed gradually at 4 °C, the volume measured, followed by filtration and analysis as described above.

The levels of RA, methyl-RA, CAA, FA and COA in plasma and urine were determined by LC-MS using an HP 1100 Series HPLC (Hewlett Packard, CA, USA) equipped with an auto injector, a binary HPLC pump, and a column heater, interfaced with an HP Series 1100 mass selective detector with an API-ES ionization chamber. The plasma extract was injected onto an HPLC column (CAPCELL PACK-MG120, 120 mm × 2.0 mm I. D., Shiseido, Tokyo, Japan) and eluted with a mixture of (A) 0.03 % formic acid in water and (B) acetonitrile as the mobile phase. Separation was achieved at a flow rate of 0.2 ml/min using a 0–50 % linear gradient of B in A (0–15 min), followed by isocratic elution with 50 % B (15–20 min). The conditions for analysis in the negative ion mode were the following: capillary voltage 4500 V, fragmentor 90 V, nebulizing pressure 40 psig, drying gas temperature 320 °C and drying gas flow 8 l/min. Data were analyzed on an HP Chem Station using selective ion monitoring and are expressed as means ± SD.

Results

Identification of RA methylated by COMT

LC-MS chromatograms of RA methylated by COMT are shown in Fig. 1. Before synthesis of methyl-RA by COMT, a peak was detected at approximately 12 min at m/z 359 in the LC-MS analysis although no peak was detected at m/z 373 corresponding to the mass number of mono-methylated RA (Fig. 1A and B). After methylation of RA by COMT, a single peak was detected at approximately 13.5 min, at m/z 373 in the LC-MS analysis that corresponded to the mass number of mono-methylated RA (Fig. 1D). This peak at m/z 373 showed the same retention time of 13.5 min in LC-MS analyses of plasma and urine following intake of PE, and therefore was identified as mono-methylated RA on the basis of the similarity in molecular weights (Fig. 1F).

European Journal of Nutrition (2005) Vol. 44, Number 1

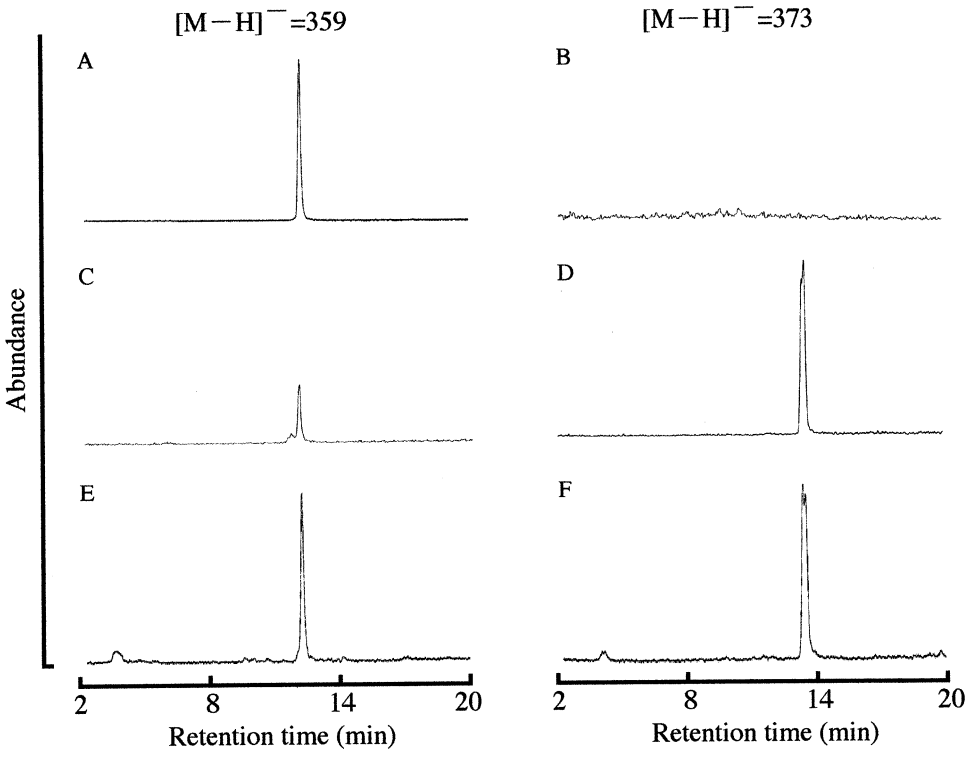

Fig. 1 Typical LC-MS chromatograms of rosmarinic acid (RA) methylated by catechol-*O*-methyltransferase (COMT) at m/z 359 (**A, C, E**) and 373 (**B, D, F**), that corresponds to the mass number of RA and mono methylated RA (methyl-RA), respectively. **A** and **B** are chromatograms prior to the synthesis of methyl-RA from RA by COMT. **C** and **D** are chromatograms after the synthesis of methyl-RA from RA by COMT. **E** and **F** show urine excreted after intake of PE that has been treated with sulfatase H-5 treatment

Identification of RA, CAA, FA and COA by LC-MS

Fig. 2 shows typical LC-MS chromatograms of urine obtained after intake of PE. Analysis of urine obtained after 6 h, which had been treated with sulfatase H-5, showed single peaks with the following characteristics: m/z 359 after 12 min that corresponded to the mass number and retention time of RA (Fig. 2A); m/z 179 after 5 min that corresponded to the mass number and retention time of CAA (Fig. 2B); and m/z 193 after 9 min that corresponded to the mass number and retention time of FA (Fig. 2C). In sulfatase H-5 treated urine obtained within 24 to 48 h after intake of PE a trace peak was eluted after approximately 10.5 min at m/z 163 that corresponded to the mass number and retention time of COA (Fig. 2D). No peaks with the above retention times were detected at m/z 359, 179, 193 or 163 in the LC-MS analyses in urines collected from subjects receiving placebo (data not shown).

Levels of RA and its related metabolites in plasma

Profiles of RA and its related metabolites in plasma after intake of PE are shown in Fig. 3. After intake of placebo no RA or related metabolites were detected in the plasma (data not shown). Within 0.5 h of taking the PE, total RA (free and conjugates) and total FA reached maximum levels of 1.15 ± 0.28 µmol/l and 0.36 ± 0.17 µmol/l, respectively. Total methyl-RA gradually reached a maximum level of 0.65 ± 0.07 µmol/l 2 h after intake of PE, while CAA, COA and their conjugated forms in plasma were under the detection limit of the method.

Levels of RA and its related metabolites in urine

The urinary excretion of RA and its related metabolites over the 48 hours after intake of PE is shown in Fig. 4 and Table 1. No RA or related metabolite were present in the urine after intake of placebo (data not shown). The main metabolites found in the urine following intake of RA were free and sulfoglucuronide conjugates of RA and methyl-RA, and sulfate conjugates of CAA and FA. A trace of COA was detected in the urine 24 to 48 h post-intake. The sum of RA, methyl-RA, CAA, FA and COA including conjugated forms excreted in the urine within 48 hours after intake of PE was 6.30 ± 2.17 % of the total RA dose with approximately 75 % of RA and its metabolites being excreted in the first 6 h.

Discussion

Perilla frutescens L. is used as a 'kitchen herb' especially in Japan. RA is a major polyphenolic component of *Perilla frutescens L.* and many other common herbs. Al-

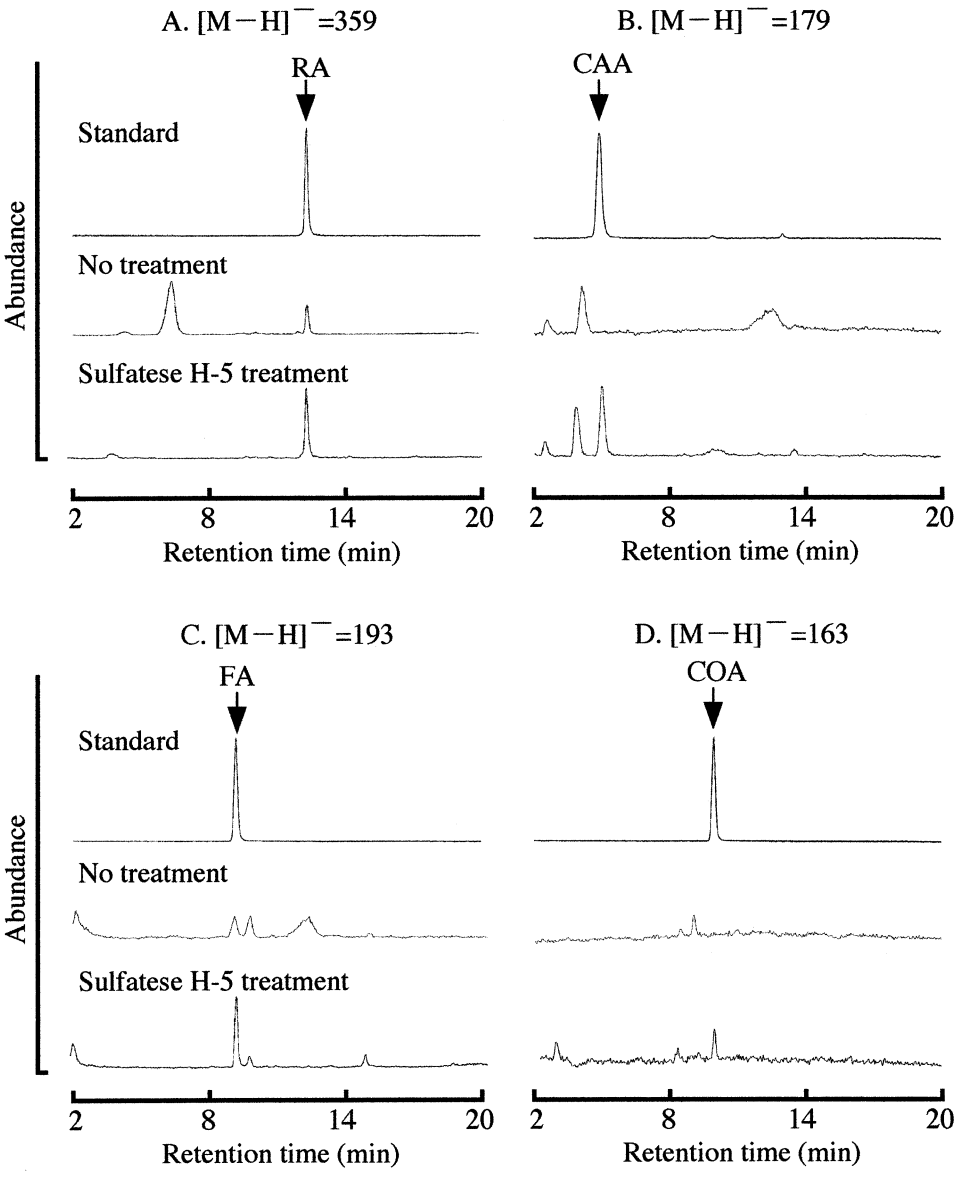

Fig. 2 Typical LC-MS chromatograms of human urine excreted after intake of perilla extract with or without sulfatase H-5 treatment. These chromatograms at m/z 359, 179, 193 and 163 show the corresponding mass number of rosmarinic acid (RA), caffeic acid (CAA), ferulic acid (FA) and *m*-coumaric acid (COA), respectively. Molecular ion $[M–H]^-$ peaks detected at about 12 min at m/z 359 were identified as RA. Molecular ion $[M–H]^-$ peaks detected at about 5 min at m/z 179 were identified as CAA. Molecular ion $[M–H]^-$ peaks detected at about 9 min at m/z 193 were identified as FA. Molecular ion $[M–H]^-$ peaks detected at about 11 min at m/z 163 were identified as COA

though RA has several physiological effects that include anti-inflammatory and antioxidative activity, there is little information regarding absorption, metabolism and urinary excretion of RA in humans. The present study had the objective of investigating these processes in healthy men, after oral administration of PE.

Previous reports showed that plasma concentration of curcuminoids and flavonoids such as curcumin, demethoxycurcumin, quercetin, catechin and luteolin reach a peak within 0.5 to 1.0 h after ingestion [23–26]. In this study, following intake of PE, RA reached a maximum concentration in the plasma by 0.5 h, followed by a gradual increase in the plasma concentration of methyl-RA that reached a peak by 2 h (Fig. 3). Approximately 75% of the total rosmarinic metabolites were then excreted in the urine within 6 h (Table 1). These results show that RA contained in PE was rapidly absorbed, subsequently methylated and then excreted in the urine.

The majority of RA and its metabolites were present in the plasma as conjugated forms (Fig. 3). Previous studies in rats and humans investigating other polyphenolic substances such as flavonoids and curcuminoids have shown that the majority of the metabolites of these compounds are also present in the plasma as conjugates and methylated forms [21, 24, 25, 27–30]. Furthermore, enzymes that catalyze these chemical transformations have been found in both human and rat tissues [21, 31–34]. These results demonstrate that ingested RA was absorbed, conjugated, and methylated in tissues, and

European Journal of Nutrition (2005) Vol. 44, Number 1

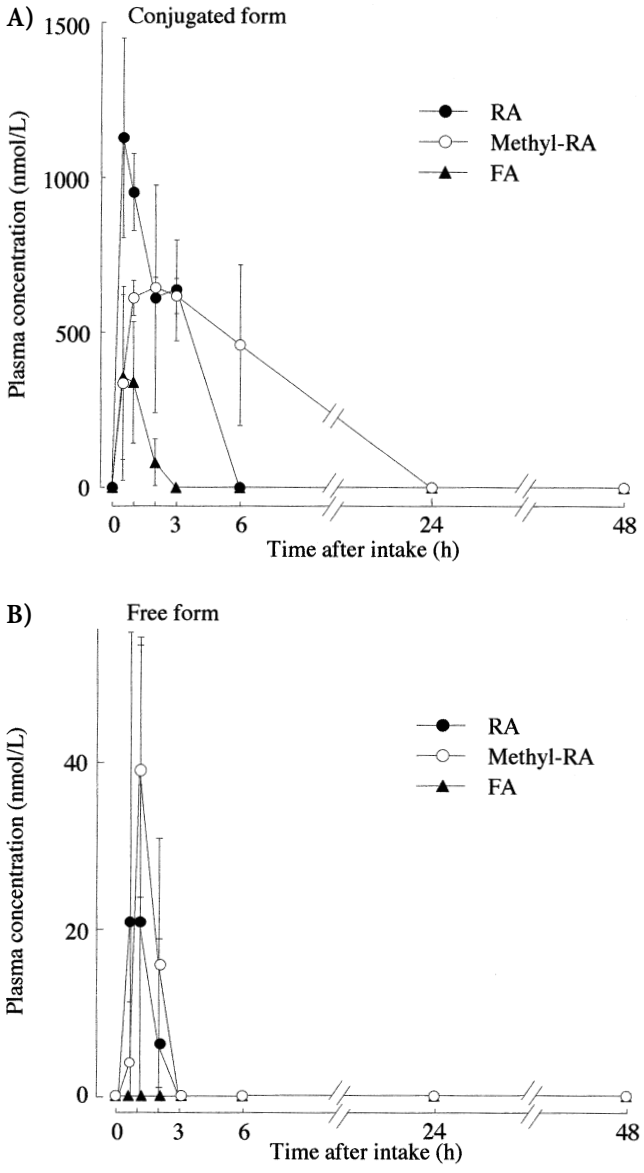

Fig. 3 Time-related changes in the plasma concentration of rosmarinic acid (RA), nomo-methylated rosmarinic acid (methyl-RA) and ferulic acid (FA) before and 0.5, 1, 2, 3, 6, 24 and 48 h after intake of PE. Data show conjugated forms (**A**) and free form (**B**) of RA, methyl-RA and FA. Values are expressed as the mean ± SD, n = 6

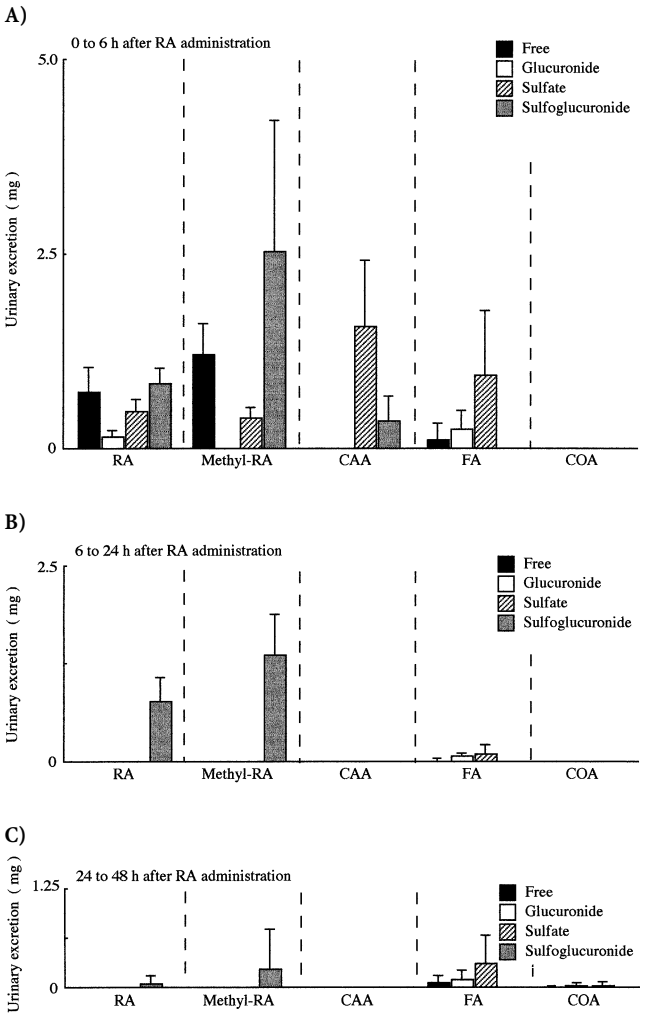

Fig. 4 Time-related changes in urine concentration of rosmarinic acid (RA), nomo-methylated rosmarinic acid (methyl-RA), caffeic acid (CAA), ferulic acid (FA) and *m*-coumaric acid (COA) after intake of perilla extract. Data show free and conjugates (glucuronide and/or sulfate) of RA, methyl-RA, CAA, FA and COA excreted in the urine from 0 to 6 h (**A**), 6 to 24 h (**B**) and 24 to 48 h (**C**) post-intake. Values are expressed as the mean ± SD, n = 6

that the majority of the ingested RA was present in plasma as metabolites.

Studies on rats receiving oral administration of RA observed that the main components excreted in the urine were RA, CAA, *m*-hydroxyphenylpropionic acid and sulfate conjugates of COA, CAA and FA [17]. Comparison of this animal study with the present study demonstrates clearly that there are large differences in the metabolites formed from RA between rats and humans. In this regard, Natsume et al. found that the chemical structure of (–)-epicatechin metabolites in plasma and urine after oral ingestion of (–)-epicatechin was also different between rats and humans [35]. Similarly, the structure of quercetin metabolites in plasma after oral ingestion of quercetin or onion (as the source of quercetin) and sulfation and glucuronidation of (–)-epicatechin in liver and intestine were also reported to be markedly different between rats and humans [32, 36, 37]. Taken together these results suggest that metabolism of polyphenolic substances such as RA varies between species due to differences in the location of enzymes, affinity of substances and characteristics of the enzymatic reactions.

In the present study the main metabolites found in the urine after intake of PE were sulfoglucuronide conjugates of RA and methyl-RA (Fig. 5). Nakazawa and Ohsawa reported that after intake of PE in humans, a glu-

Table 1 Total recovery of rosmarinic acid (RA), nomo-methylated rosmarinic acid (methyl-RA), caffeic acid (CAA), ferulic acid (FA) and *m*-coumaric acid (COA) metabolites excreted in urine after intake of perilla extract (PE)[1]

	Time after intake of PE (h)			
	0 to 6	6 to 24	24 to 32	0 to 48
Ratio to RA intake (%)				
RA	1.09±0.25	0.38±0.15	0.02±0.05	1.50±0.35
Methyl-RA	2.07±1.00	0.68±0.26	0.11±0.15	2.86±1.05
CAA	0.96±0.51	ND	ND	0.96±0.51
FEA	0.64±0.36	0.09±0.08	0.22±0.21	0.96±0.30
COA	ND	ND	0.02±0.03	0.02±0.03
Total[2]	4.76±1.58	1.15±0.94	0.38±0.36	6.30±2.17

[1] Means ± SD; n = 6; ND, not detected; Total RA, methyl-RA, CAA, FA and COA metabolites mean the sum of free, glucuronide, sulfate and sulfoglucuronide conjugates

[2] Sum of RA, methyl-RA, CAA, FEA and COA

curonide conjugate of 1-*O*-(2, 4, 5-trimethoxycinnamoyl) may be produced as a result of hydrolytic cleavage of RA in the digestive tract and that this metabolite was detectable in both plasma and urine. According to results of experiments using Caco-2 as a model of a human intestinal cell line, glucuronide and/or sulfate conjugates of flavone, flavonol and isoflavone may be present in intestinal epithelium [38] and may be produced as a result of hydrolytic cleavage of RA in the digestive tract. It has also been reported that production of UGT1A, one of the isoforms of UDP-glucuronosyl transferase, is enhanced by the addition of quercetin to Caco-2 cells [34]. Vaidyanathan and Walle reported that catechin was sulfated by sulfotransferase 1A1 in human liver and intestine, and suggested that sulfation, rather than glucuronidation, in these two organs was the main route of (–)-epicatechin metabolism in humans [32]. These results indicate that conjugation of polyphenolic substances either by glucuronidation and/or sulfation occurs in human tissues such as the intestine and liver. As shown by the scheme in Fig. 5, ingested RA may also be absorbed, conjugated and methylated in the human intestine and liver, and excreted in urine as sulfoglucuronide conjugates of RA and methyl-RA.

We also observed that after administration of PE, sulfate conjugates of CAA and FA were detected in the urine within 0 to 6 h, while trace amounts of sulfate and/or glucuronide conjugate of COA were excreted 24 to 48 h post-intake (Fig. 4). This result implies cleavage of ingested RA occurs in human tissue, although the main site of this degradation remains unclear. Plumb et al. reported that while esterase activity was not observed in human intestinal epithelium, liver or plasma, activity was observed in colonic microflora [39]. Moreover, mi-

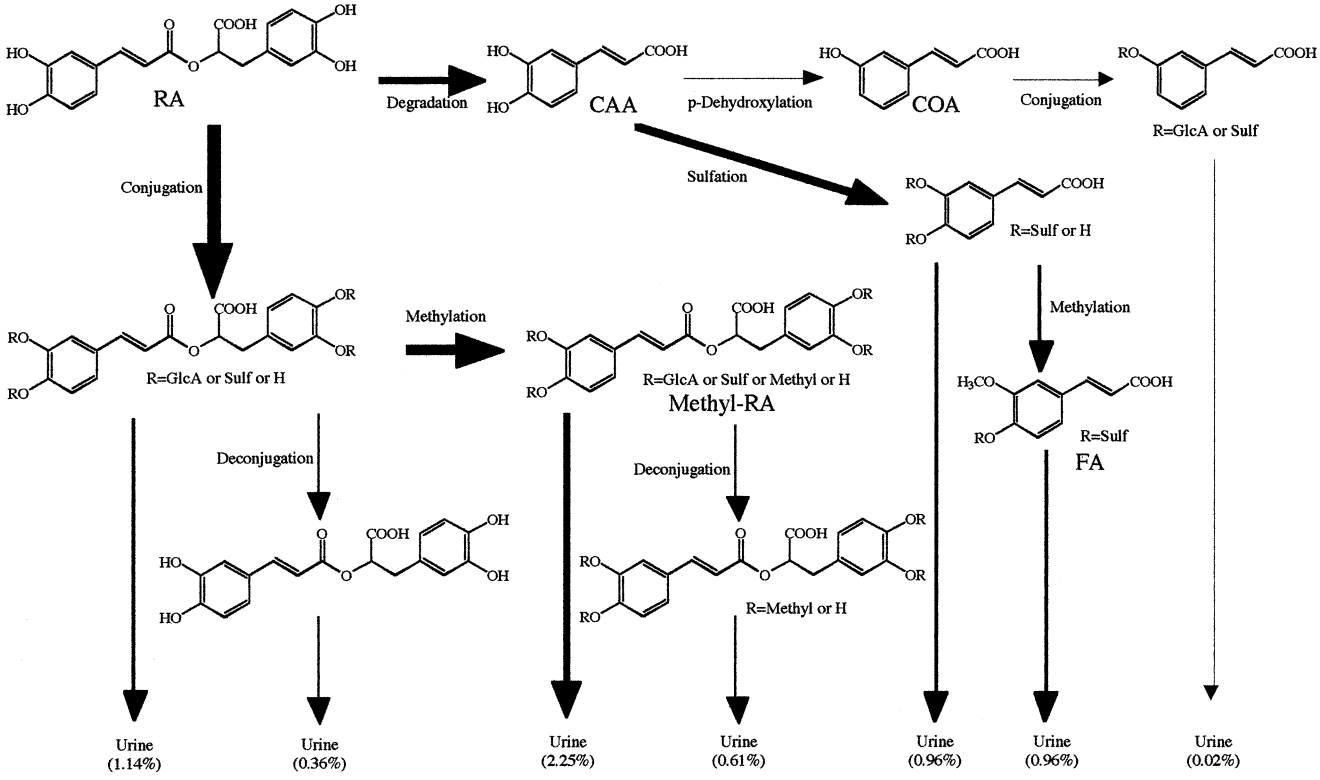

Fig. 5 Proposed scheme of possible metabolic fate in healthy men of ingested rosmarinic acid (RA) contained in perilla extract. The bold arrow shows the major pathway. *CAA* caffeic acid; *FA* ferulic acid; *COA* coumaric acid; *Methyl-RA* nomo-methylated rosmarinic acid; *GlcA* glucuronide moiety; *Sulf* sulfate moiety

croflora in the digestive tract may cause biotransformation of hydroxycinnamates by *p*-dehydroxylation [40]. Based on these results we hypothesize a scheme for RA metabolism in humans (Fig. 5) that involves microbial esterase in the digestive tract hydrolyzing the ester linkages in RA with only minimal *p*-dehydroxylation of CAA occurring in the lower portion of the tract. The resulting CAA and COA derived from RA are then absorbed, conjugated and methylated in tissues such as the digestive tract and liver resulting in a variety of metabolites such as glucuronidated or sulfated conjugates of CAA, FA and COA.

As shown in Figs. 3 and 4, free forms of RA and methyl-RA were minor components in the plasma, whereas considerable quantities of free forms of RA and methyl-RA were excreted in the urine. We have reported previously that while high levels of non-conjugated catechin were present in human urine after ingestion of chocolate and cocoa, rich in (–)-epicatechin, plasma contained only trace amounts of this non-conjugated metabolite [22]. A similar pattern of distribution has also been observed in rats after oral administration of FA [41]. These results suggest that deconjugation of metabolites may occur in the renal pathway catalysed by β-glucuronidase located in the kidney [42]. The physiological significance of this deconjugation, however, remains unclear.

In conclusion, this study demonstrated that ingested RA was absorbed and metabolized into conjugated and/or methylated forms. A portion of RA was also metabolized and degraded as conjugates of CAA, FA and COA.

Polyphenol substances in foods have been shown to have various *in vivo* and *in vitro* physiological effects that have the potential to have a beneficial contribution to human health. There is evidence that polyphenolic substances such as flavonoids and phenolic acids are degraded by gut flora and then metabolized in various tissues such as intestine, liver and kidney [43, 44]. Accordingly, when investigating the physiological functions of polyphenolic substances, it is very important to consider the metabolism of these components *in vivo*.

References

1. Makino T, Ono T, Muso E, Honda G (1998) Inhibitory effect of Perilla frutescens and its phenolic constituents on cultured murine mesangial cell proliferation. Planta Med 64:541–545
2. al-Sereiti MR, Abu-Amer KM, Sen P (1999) Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. Indian J Exp Biol 37:124–130
3. Areias F, Valentao P, Andrade PB, Ferreres F, Seabra RM (2000) Flavonoids and phenolic acids of sage: influence of some agricultural factors. J Agric Food Chem 48:6081–6084
4. Ellis BE, Towers GH (1970) Biogenesis of rosmarinic acid in Mentha. Biochem J 118:291–297
5. Tada H, Murakami Y, Omoto T, Shimomura K, Ishimaru K (1996) Rosmarinic acid and related phenolics in hairy root cultures of ocimum basilicum. Phytochemistry 42:431–434
6. Dapkevicius A, van Beek TA, Lelyveld GP, et al. (2002) Isolation and structure elucidation of radical scavengers from Thymus vulgaris leaves. J Nat Prod 65: 892–896
7. Hollman PC, Feskens EJ, Katan MB (1999) Tea flavonols in cardiovascular disease and cancer epidemiology. Proc Soc Exp Biol Med 220:198–202
8. Renaud S, De Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523–1526
9. Nakamura Y, Ohto Y, Murakami A, Ohigashi H (1998) Superoxide scavenging activity of rosmarinic acid from Perilla frutescens Britton Var. Acuta F. viridis. J Agric Food Chem 46:4545–4550
10. Fuhrman B, Volkova N, Rosenblat M, Aviram M (2000) Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. Antioxid Redox Signal 2:491–506
11. Frankel EN, Huang S-W, Aeschbach R, Prior E (1996) Antioxidant Activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. J Agric Food Chem 44:131–135
12. Kimura Y, Okuda H, Okuda T, Hatano T, Arichi S (1987) Studies on the activities of tannins and related compounds, X. Effects of caffeetannins and related compounds on arachidonate metabolism in human polymorphonuclear leukocytes. J Nat Prod 50:392–399
13. Englberger W, Hadding U, Etschenberg E, et al. (1988) Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory activity. Int J Immunopharmacol 10:729–737
14. Ito H, Miyazaki T, Ono M, Sakurai H (1998) Antiallergic activities of rhabdosiin and its related compounds: chemical and biochemical evaluations. Bioorg Med Chem 6:1051–1056
15. Osakabe N, Yasuda A, Natsume M, et al. (2002) Rosmarinic acid, a major polyphenolic component of Perilla frutescens, reduces lipopolysaccharide (LPS)-induced liver injury in D-galactosamine (D-GalN)-sensitized mice. Free Radic Biol Med 33:798–806
16. Sanbongi C, Takano H, Osakabe N, et al. (2003) Rosmarinic acid inhibits lung injury induced by diesel exhaust particles. Free Radic Biol Med 34:1060–1069
17. Nakazawa T, Ohsawa K (1998) Metabolism of rosmarinic acid in rats. J Nat Prod 61:993–996
18. Ritschel WA, Starzacher A, Sabouni A, Hussain AS, Koch HP (1989) Percutaneous absorption of rosmarinic acid in the rat. Methods Find Exp Clin Pharmacol 11:345–352
19. Nakazawa T, Ohsawa K (2000) Metabolites of orally administered Perilla frutescens extract in rats and humans. Biol Pharm Bull 23:122–127
20. Spencer JPE, Schroeter H, Kuhnle G, et al. (2001) Epicatechin and its in vivo metabolite, 3'-O-methyl epicatechin, protect human fibroblasts from oxidative-stress-induced cell death involving caspase-3 activation. Biochem J 354:493–500
21. Piskula MK, Terao J (1998) Accumulation of (–)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rat tissues. J Nutr 128: 1172–1178

22. Baba S, Osakabe N, Yasuda A, et al. (2000) Bioavailability of (–)-epicatechin upon intake of chocolate and cocoa in human volunteers. Free Radic Res 33:635–641
23. Piskula MK, Terao J (1998) Quercetin's solubility affects its accumulation in rat plasma after oral administration. J Agric Food Chem 46:4313–4317
24. Shimoi K, Okada H, Furugori M, et al. (1998) Intestinal absorption of luteolin and luteolin 7-O-beta-glucoside in rats and humans. FEBS Lett 438:220–224
25. Asai A, Miyazawa T (2000) Occurrence of orally administered curcuminoid as glucuronide and glucuronide/sulfate conjugates in rat plasma. Life Sci 67: 2785–2793
26. Baba S, Osakabe N, Natsume M, Muto Y, Takizawa T, Terao J (2001) In vivo comparison of the bioavailability of (+)-catechin, (–)-epicatechin and their mixture in orally administered rats. J Nutr 131:2885–2891
27. da Silva EL, Piskula MK, Yamamoto N, Moon JH, Terao J (1998) Quercetin metabolites inhibit copper ion-induced lipid peroxidation in rat plasma. FEBS Lett 430:405–408
28. Erlund I, Meririnne E, Alfthan G, Aro A (2001) Plasma kinetics and urinary excretion of the flavonones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. J Nutr 131:235–241
29. Sesink AL, O'Leary KA, Hollman PC (2001) Quercetin glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4'-glucoside. J Nutr 131:1938–1941
30. Setchell KD, Brown NM, Zimmer-Nechemias L, et al. (2002) Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J Clin Nutr 76: 447–453
31. Galijatovic A, Walle UK, Walle T (2000) Induction of UDP-glucuronosyltransferase by the flavonoids chrysin and quercetin in Caco-2 cells. Pharm Res 17: 21–26
32. Vaidyanathan JB, Walle T (2002) Glucuronidation and sulfation of the tea flavonoid (–)-epicatechin by the human and rat enzymes. Drug Metab Dispos 30:897–903
33. Walle UK, Walle T (2002) Induction of Human UDP-Glucuronosyltransferase UGT1A1 by Flavonoids-Structural Requirements. Drug Metab Dispos 30: 564–569
34. Boersma MG, van der Woude H, Bogaards J, et al. (2002) Regioselectivity of phase II metabolism of luteolin and quercetin by UDP-glucuronosyl transferases. Chem Res Toxicol 15:662–670
35. Natsume M, Osakabe N, Oyama M, et al. (2003) Structures of (–)-epicatechin glucuronide identified from plasma and urine after oral ingestion of (–)-epicatechin: differences between human and rat. Free Radic Biol Med 34: 840–849
36. Moon J, Tsushida T, Nakahara K, Terao J (2001) Identification of quercetin 3-O-beta-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. Free Radic Biol Med 30:1274–1285
37. Day AJ, Mellon F, Barron D, Sarrazin G, Morgan MR, Williamson G (2001) Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. Free Radic Res 35:941–952
38. Murota K, Shimizu S, Miyamoto S, et al. (2002) Unique uptake and transport of isoflavone aglycones by human intestinal caco-2 cells: comparison of isoflavonoids and flavonoids. J Nutr 132:1956–1961
39. Plumb GW, Garcia MT, Kroon PA, Rhodes M, Ridley S, Williamson G (1999) Metabolism of chlorogenic acid by human plasma, liver, intestine and gut microflora. J Sci Food Agric 79: 390–392
40. Goodwin BL, Ruthven CR, Sandler M (1994) Gut flora and the origin of some urinary aromatic phenolic compounds. Biochem Pharmacol 47:2294–2297
41. Rondini L, Peyrat-Maillard MN, Marsset-Baglieri A, Berset C (2002) Sulfated ferulic acid is the main in vivo metabolite found after short-term ingestion of free ferulic acid in rats. J Agric Food Chem 50:3037–3041
42. Rodriguez-Cuartero A, Barcelona-Martin F, Perez-Blanco FJ (2000) Urinary beta-glucuronidase in renal cell carcinoma. Clin Nephrol 53:288–290
43. Williamson G, Day AJ, Plumb GW, Couteau D (2000) Human metabolic pathways of dietary flavonoids and cinnamates. Biochem Soc Trans 28:16–22
44. Rechner AR, Kuhnle G, Bremner P, Hubbard GP, Moore KP, Rice-Evans CA (2002) The metabolic fate of dietary polyphenols in humans. Free Radic Biol Med 33:220–235