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Modulation of hepatic phase II phenol sulfotransferase and antioxidant status by phenolic acids in rats☆

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Abstract

Phenolic acids have significant biological and pharmacological properties and some have demonstrated remarkable ability to alter sulfate conjugation. However, the modulatory effects of phenolic acids on phenol sulfotransferases (PSTs) in vivo have not been described. The present investigation evaluates the role of phenolic acid on the expression of PSTs in male Sprague–Dawley rat liver. According to the results, gentisic acid, gallic acid and *p*-coumaric acid in a dosage of 100 mg/kg of body weight for 14 consecutive days significantly increased P-form PST (PST-P) activity as compared with that of the control rats ($P<.05$), whereas the activity of M-form PST (PST-M) in rats that received gallic acid and *p*-coumaric acid were also significantly ($P<.05$) higher than in the control rats. Reverse transcriptase–polymerase chain reaction results indicated that the changes in both PST-P and PST-M mRNA levels by phenolic acids were similar to those noted in the enzyme activity levels. The plasma obtained from phenolic acid-administered rats had significantly ($P<.05$) increased oxygen radical absorbance capacity ($ORAC_{ROO\cdot}$) values than that from control rats. In a bioavailability study, following oral administration of gallic acid and *p*-coumaric acid, the phenolic acids were detected in the plasma, and the C_{max} values after 2.0-h administration were 665±23 and 550±33 nmol/L, respectively. There was a significant correlation between the activity of both forms of PSTs and the antioxidant capacity of $ORAC_{ROO\cdot}$ value by phenolic acids ($r=.74$, $P<.05$ and $r=.77$, $P<.05$). These data suggest that phenolic acids might alter sulfate conjugation and antioxidant capacity in living systems.

Keywords: Phenolic acids; Phenol sulfotransferase; Antioxidant capacity; Bioavailability; Sprague–Dawley rat

1. Introduction

Chemoprevention is one of the most promising areas in cancer research. Potential chemopreventive agents may function by a variety of mechanisms directed at all major stages of carcinogenesis. One proposed mechanism for cellular protection, against the chemical and neoplastic effects of carcinogens, involves the induction of phase II detoxification enzymes [1]. A large body of evidence, based on preclinical and clinical research, also indicates that modulation of the body's phase II detoxification enzymes could provide an effective approach for cancer prevention [2].

The phenol sulfotransferases (PSTs) are the main phase II sulfoconjugation enzyme for catecholamines, thyroid hormones and drugs, thereby facilitating biliary or urinary excretion and detoxification [3]. Sulfoconjugation plays not only an important role in xenobiotic metabolism, but also a critical role in steroid biosynthesis and in modulating the biological activity and facilitating the inactivation and elimination of potent endogenous chemicals, including steroids, catecholamines and thyroid hormones. Regulation of PSTs by different inducers has been relatively well studied [4]. Rat is one of the most well-studied species for PSTs. Two major PSTs isoforms are dominant in rats: PST, which catalyzes the sulfation of phenols, and hydroxysteroid sulfotransferase, which catalyzes the sulfation of alcohols [5]. Hepatic phenol and hydroxysteroid sulfotransferase

Abbreviations: GST, glutathione *S*-transfersase; $ORAC_{ROO\cdot}$, oxygen radical absorbance capacity; β-PE, β-phycoerythrin; PST-P, P-form phenol sulfotransferases; PST-M, M-form phenol sulfotransferase; RT-PCR, reverse transcriptase–polymerase chain reaction; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gels; TEAC, Trolox equivalent antioxidant capacity.

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show a distinct sex dimorphism in rats [6]. Higher immunoreactivity of phenol and hydroxysteroid sulfotransferase proteins has been shown in livers of male and female rats, respectively. Therefore, measurements of the induction of PST activity may provide an efficient approach to understanding the chemopreventive mechanisms of dietary compounds.

Phenolic acids are a group of polyphenols that are widely distributed in the diet, mostly in whole grains, fruits, vegetables and beverage [7]. These phenolic compounds exhibit good in vitro antioxidant and chemoprotective properties, which may have some beneficial effects in vivo [8]. The extent of their protective effect in vivo depends on their bioavailability [9]. Some naturally occurring phenolic acid and analogues, namely, ferulic acid and gallic acid, are known to display a wide variety of biological functions in addition to their primary antioxidant activity, which are mainly related to modulation of carcinogenesis [10]. Previous studies in our laboratory have shown that *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid and *p*-coumaric acid could modulate phase II sulfate conjugative enzyme. These phenolic acids were also found to possess antioxidant capacity in the oxygen radical absorbance capacity ($ORAC_{ROO\cdot}$) and Trolox equivalent antioxidant capacity (TEAC) assays [11]. Moreover, the activity of P-form PST (PST-P) could be promoted by the combinations of all those phenolic acids [12]. The overall effects of phenolic acids on the activity of PST-P are highly correlated to their $ORAC_{ROO\cdot}$ values, suggesting that antioxidant phenolic acids might alter sulfate conjugation. However, there is little information in the literature on the induction of PSTs by phenolic acid in vivo system.

In this study, rats were orally administered *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, *p*-coumaric acid or gallic acid, and the effects of these active components on hepatic PST activities and mRNA expressions were investigated. Moreover, the antioxidant status as reflected by the $ORAC_{ROO\cdot}$ in plasma of rats exposed to the various phenolic acids was also determined. Each of the phenolic acids (gallic acid or *p*-coumaric acid) was further orally administered to rats to evaluate the absorption behavior.

2. Materials and methods

2.1. Materials

p-Hydroxybenzoic acid, gentisic acid (2,5-dihydroxybenzoic acid, 98% purity), ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid, 99% purity), gallic acid (3,4,5-trihydroxybenzoic acid, 98% purity), *p*-coumaric acid (*trans*-4-hydroxycinnamic acid), *p*-nitrophenol, dopamine, sucrose and Na_2EDTA were obtained from Sigma Chem (St. Louis, MO); [^{35}S]-labeled 3′-phosphoadenosine-5′-phosphosulfate ($PAPS^{35}$) (1.0–1.5 Ci/mmol) was obtained from DuPont New England Nuclear (Boston, MA); a TRIzol RNA isolation kit was obtained from Life Technologies (Rockville, MD); and primers for reverse transcriptase–polymerase chain reaction (RT-PCR), dNTP, reverse transcriptase and Taq polymerase were obtained from Gibco BRL (Cergy Pontoise, France). Protein assay reagent was purchased from Bio-Rad. All other chemicals used were of the highest pure grade available.

2.2. Animal treatment

Male Sprague–Dawley rats (200±10 g; obtained from the National Animal Breeding and Research Center, Taipei, Taiwan) were used for the experiments. Animals had free access to water and diet, which is a casein-based phenolic acid-free diet. The diet contained (in g/kg) casein (200), corn oil (50), sucrose (500), corn starch (150), cellulose (50), AIN-76 vitamin mix (10), AIN-76 mineral mix (35), methionine (3) and choline bitartrate (2). All diet ingredients were supplied by Harlan Teklad (Madison, WI). Food intake and body weight were measured daily. After an adaptation period for 1 week, the rats were divided at random into six groups (six rats per group). To study the effects of phenolic acids on the induction of phase II PSTs in rats, phenolic acid (*p*-hydroxybenzoic acid, gentisic acid, gallic acid, ferulic acid, and *p*-coumaric acid) was given daily by gavage to the animals at a dosage of 100 mg/kg body weight per day for 14 consecutive days. Before injection, the compound was dissolved in propylene glycol and then mixed with saline (the concentration of propylene glycol being less than 1%). The control group was treated with vehicle alone. At the end of experimental, the rats were sacrificed by an overdose of the diethyl ether 2 h after the last treatment and subjected to the following analytical procedures: their livers were collected, washed with sterile ice-cold NaCl (0.9%) solution and kept in dry ice bath. Samples were stored at −80°C until use (not more than 1 week). All experimental procedures involving animals were conducted in accordance with the National Institutes of Health guidelines. This experiment was approved by the Institutional Animal Care and Use Committee of the National Chung Hsing University, Taichung, Taiwan.

2.3. Cytosol preparations

Immediately after being removed from each animal, the liver was rinsed extensively in cold phosphate-buffered saline (PBS). The tissues were then cut into small pieces using scissors, rinsed twice with cold PBS, followed by homogenization in ice-cold 50 mM phosphate buffer containing 2 mM EDTA on ice. The homogenates were centrifuged at 10,000×*g* for 15 min at 4°C. Cytosol aliquots were collected and preserved at −80°C for enzymatic assay.

2.4. Assay of PST activity

The induced PST activity, by phenolic acids, was determined using the PST assay [11]. Rat liver cytosols (100 μg) were used as the enzyme source in a total reaction volume of 200 μl of reaction mixture contained 110 μl of

0.1 M potassium phosphate buffer (pH 7.0), 30 μl of the liver cytosols and 30 μl of the substrate; 30 μl [^{35}S]-labeled PAPS (final concentration, 6.7 μM) was added, at successive intervals, to tubes at 37°C in a water bath, and the reaction was terminated after 20 min by addition of 0.1 M barium acetate (200 μl). Any unreacted PAPS, free sulfate or protein was precipitated by two additions of 0.1 M barium hydroxide (200 μl), followed by 0.1 M zinc sulfate (200 μl). After centrifugation (11,500×*g* for 3 min), 500 μl of the supernatant was thoroughly mixed with 4 ml scintillant, and the radioactivity measured by liquid scintillation spectrometry. The protein content of the liver cytosols was determined using a Bio-Rad protein assay kit, and PST activity was expressed as picomoles per minute per milligram of protein. All samples were assayed, in triplicate, in three independent experiments.

2.5. RNA extraction and RT-PCR

RT-PCR was performed to determine the level of PST gene expression. Total RNA from rat liver tissues was isolated using the TRIzol RNA isolation kit (Life Technologies, Rockville, MD) as described in the manufacturer's manual. cDNA was synthesized with random primers using the Reverse Transcription system (Promega, Madison, WI) according to the manufacturer's instructions. The concentration and purity of the extracted RNA were checked spectrophotometrically by measuring 260/280 absorption ratios. PST-P was amplified at 94°C for 45 s, 56°C for 35 s and 72°C for 45 s for a total of 30 cycles followed by a 10-min extension at 72°C using the following primers: 5′-GTG TCC TAT GGG TCG TGG TA- 3′ and 5′-TTC TGG GCT ACA GTG AAG GTA-3′. M-form PST (PST-M) was amplified at 94°C for 30 s, 58°C for 30 s and 72°C for 1 min for a total of 30 cycles followed by a 10-min extension at 72°C using the following primers: 5′-TCC TCA AAG GAT ATG TTC CG-3′ and 5′-CAG TTC CTT CT C CAT GAG AT-3′. As a housekeeping gene, rat β-actin was amplified at 94°C for 45 s, 55°C for 45 s and 72°C for 45 s for a total of 28 cycles followed by a 10-min extension at 72°C using the following primers: 5′-GAT GTA CGT AGC CAT CCA-3′ and 5′-GTG CCA ACC AGA CAG CA-3′. The specificity of all primers was tested using the BLAST from the National Center for Biotechnology Information Open Reading Frame software. Amplification products were resolved by electrophoresis on a 1.8% agarose gel containing 0.06 mg/L ethidium bromide. The gel was then photographed under ultraviolet transillumination. For quantification, the PCR bands on the photograph of the gel were scanned using a densitometer linked to a computer analysis system. Net band intensity (background-subtracted intensity) was normalized to values for β-actin and plotted as arbitrary units. Water samples or RNA samples containing no RT were amplified in parallel to ensure that no contaminating DNA was present during PCR.

2.6. Assessment of the plasma antioxidant status

The $ORAC_{ROO\cdot}$ assay was based on the procedure described by Cao and Prior [13]. Free radicals were produced by AAPH, and the oxidation of the fluorescent indicator protein β-phycoerythrin (β-PE) was measured. Both reagents were prepared in 75 mM phosphate buffer (pH 7.4), and 50 μM Trolox was used as standard. Blood samples were collected in tubes containing 2% sodium heparin at 2 h after supplement administration. Each tube was centrifuged at 1000×*g* for 5 min, and the plasma was immediately placed in an ice bath until appropriately diluted with phosphate buffer (pH 7.4) before the $ORAC_{ROO\cdot}$ assay. To measure the $ORAC_{ROO\cdot}$ in the nonprotein fraction, the plasma was treated with 0.25 M perchloric acid and centrifuged at 15,000 rpm for 15 min at 4°C. Then, the resultant supernatants were stored at −80°C prior to analysis. The reaction was performed in 96-well microtiter plates and consisted of 170 μl of β-PE (80 mg/L) and 10 μl of diluted plasma incubated at 37°C for 15 min. The reaction was initiated by the addition of 20 μl of AAPH (240 μM), and the fluorescence (emission, 565 nm; excitation, 540 nm) was recorded every 5 min until reading had declined to less than 5% of initial reading. The $ORAC_{ROO\cdot}$ values were calculated and expressed as micromoles of Trolox equivalent per liter.

2.7. Determination of phenolic acids in rat plasma

After overnight food deprivation, 30 Sprague–Dawley rats were randomly assigned to two groups (15 rats per group) and then were orally administered gallic acid or *p*-coumaric acid (100 mg/kg body weight per day in 1% propylene glycol) by gastric intubation. Three rats per one group were killed at 0.5, 1.0, 2.0 and 4.0 h postadministration by using a heparinized needle and syringe under anesthesia with diethyl ether in the inferior vena cava. Plasma was immediately prepared by centrifugation at 1000×*g* for 15 min at 4°C and stored at −80°C until use. The identification and quantification of the phenolic acids were analyzed by HPLC (Hitachi, Japan), using the LiChrosphere RP-18 column (150×4 mm^2, 5 μm) and photodiode array detector (measured at 280 nm). To 25 μl of plasma were added 25 μl of 0.1 M sodium acetate buffer (pH 5.0) and 450 μl of 0.83 M acetic acid in methanol. The mixture was vortexed for 30 s, sonicated for 30 s and finally centrifuged at 8500×*g* for 5 min at 4°C. Elution was carried out at room temperature and utilized 2% (v/v) acetic acid in water as solvent A and 0.5% acetic acid in water and acetonitrile (50:50, v/v) as solvent B. The elution gradient program was as follows: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min) at a flow rate of 1 ml/min. The injection volume for standards sample extracts was 20 μl. Two phenolic acids (gallic acid and *p*-coumaric acid) were quantified using the external standard method. Quantification was based on peak area. Calibration curves of the standards were made by diluting stock standards in

methanol to yield 0–300 mg/L (phenolic acids). Linear regression was fitted to the data to obtain regression coefficients >.99 for phenolic acid standard curves.

2.8. Statistical analysis

Correlation and regression analysis and principal component analysis were performed using the SigmaPlot scientific graph system. The component loading included $ORAC_{ROO\cdot}$ activity as well as activities of PST-P and PST-M. Each experiment was performed in triplicate and repeated three times. The results were expressed as means± S.E.M. All data were analyzed by one-way analysis of variance, with post hoc comparisons by Student–Newman–Keuls test. Difference was considered significant when the P values were <.05.

3. Results

3.1. Effects of phenolic acids on the relative tissue weight of rats

Table 1 shows the body weight, liver weight and relative liver tissue weight of rats in each group. There was no difference among the relative liver weight of rats with gavage of phenolic acids (100 mg/kg of body weight) for 14 consecutive days when compared to control. All rats remained in good health throughout the experimental period. Body weight gains did not differ among the test and the control groups.

3.2. PST-P and PST-M activities in rat liver following phenolic acid treatment

After 2 weeks of treatment, the growth rate of the rats did not change from each phenolic acid as compared with the control. The effects of phenolic acids on the hepatic phase II PST activities are shown in Fig. 1. No change in the PST-P activity was noted in rats administered with either *p*-hydroxybenzoic acid or ferulic acid as compared with that in the control rats. Rats that received 100 mg/kg of gentisic acid, *p*-coumaric acid or gallic acid had higher PST-P activities than the control (P<.05) (Fig. 1A). To measure the PST-M activity, dopamine was used as the substrate for the enzyme activity assay instead of *p*-nitrophenol. As the results indicated, *p*-coumaric acid and gallic acid significantly (P<.05) increased hepatic PST-M activity as compared to that of the control rats (Fig. 1B). Gentisic acid and ferulic acid were relatively less effective than either gallic acid or *p*-coumaric acid in increasing liver PST-M activity. On the other hand, treatment of rat with *p*-hydroxybenzoic acid resulted in a slight induction in the activity of this enzyme.

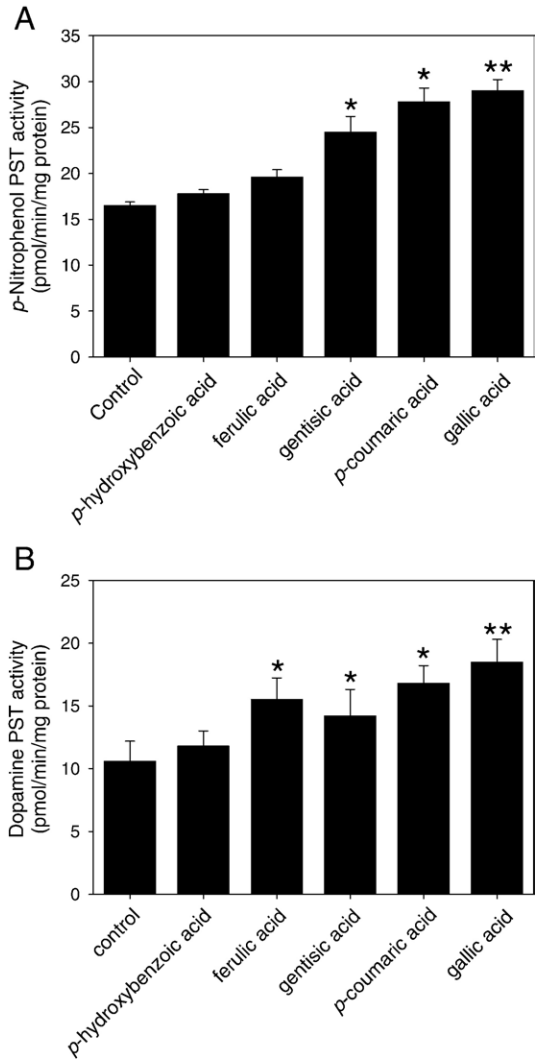

Fig. 1. Effect of phenolic acids on (A) *p*-nitrophenol and (B) dopamine PST activity in rat livers. Enzyme activity was determined by the radioactive assay method using 250 μg of liver cytosolic protein. Results are presented as means±S.E.M. (n=6). *P<.05 and **P<.01, significantly different from the control value.

Table 1
Effects of phenolic acids (*p*-hydroxybenzoic acid, ferulic acid, gentisic acid, gallic acid and *p*-coumaric acid) on body weights, liver weights and relative liver weights of rats

	Body weight (g)[a]	Liver weight (g)[a]	Relative liver weights
Control	252±5	8.5±0.5	3.2±0.4
p-HA (0.1 g/kg body weight)	256±3	8.2±0.2	3.2±0.2
FA (0.1 g/kg body weight)	255±7	8.3±0.3	3.3±0.1
GEA (0.1 g/kg body weight)	263±8	8.4±0.5	3.1±0.3
GA (0.1 g/kg body weight)	265±4	8.6±0.3	3.2±0.3
p-CA (0.1 g/kg body weight)	253±4	8.2±0.2	3.2±0.3

[a] Rats were orally administered with 100 mg/kg of control, *p*-hydroxybenzoic acid (*p*-HA), ferulic acid (FA), gentisic acid (GEA), gallic acid (GA) and *p*-coumaric acid (*p*-CA) for 2 weeks. Control rats received vehicle solution. Data are expressed as mean±SD from six rats.

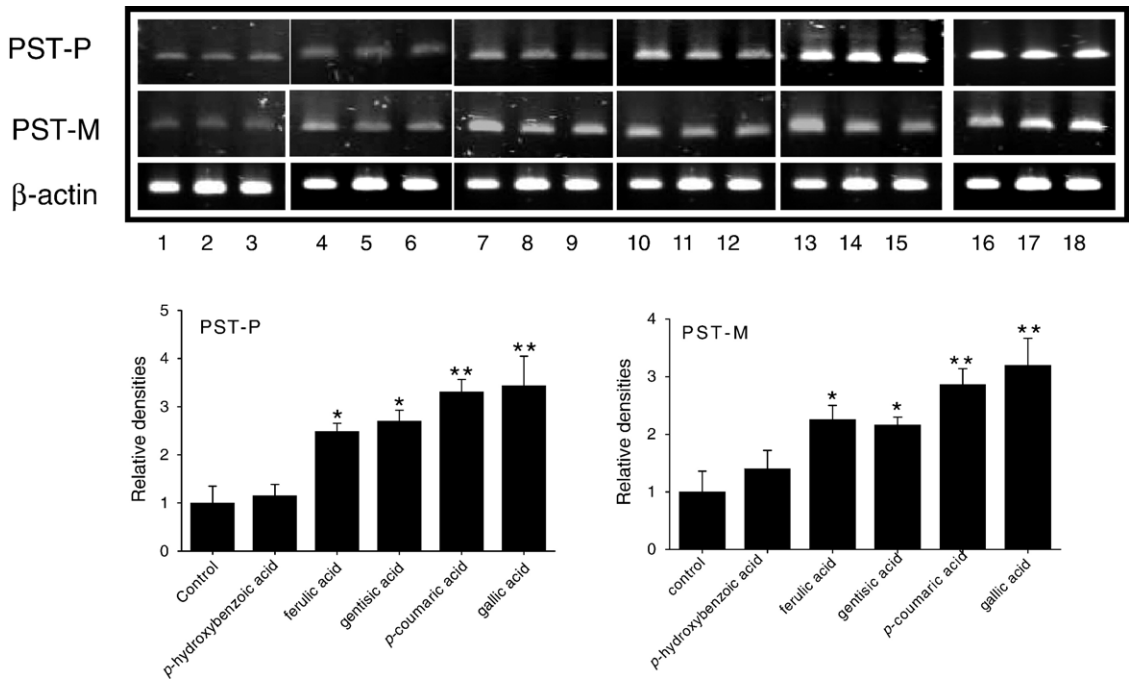

Fig. 2. RT-PCR analysis for the expression of PST-P and PST-M in the liver of control and phenolic acid-treated rats. Lanes 1–3, control; lanes 4–6, 0.1 g/kg per day of *p*-hydroxybenzoic acid; lanes 7–9, 0.1 g/kg per day of ferulic acid; lanes 10–12, 0.1 g/kg per day of gentisic acid; lanes 13–15, 0.1 g/kg per day of *p*-coumaric acid; lanes 16–18, 0.1 g/kg per day of gallic acid. Total RNA was extracted using TRIzol as described in Materials and Methods. A 20-μg aliquot of RNA of each sample was subjected to RT-PCR analysis. The RT-PCR products were separated on 1.8% agarose gel and digitally imaged after staining with ethidium bromide. The division factors are plotted and expressed as relative densities. $*P<.05$; $**P<.01$.

3.3. mRNA expression of PST-P and PST-M in rat liver following phenolic acid treatment

To further examine whether PST mRNA expression was modulated by phenolic acids, an RT-PCR analysis was performed. As shown in Fig. 2, PST-P and PST-M mRNA expression in liver tissues were simultaneously modulated by phenolic acids. For PST-P, gallic acid had the greater potency in induction of mRNA than *p*-coumaric acid, and *p*-coumaric acid was greater than gentisic acid and ferulic acid. As quantitated by densitometry, the induction of PST-P by ferulic acid, gentisic acid, *p*-coumaric acid and gallic acid was 2.4-, 2.7-, 3.3- and 3.6-fold, respectively, as compared to those of the control rats. The increase of

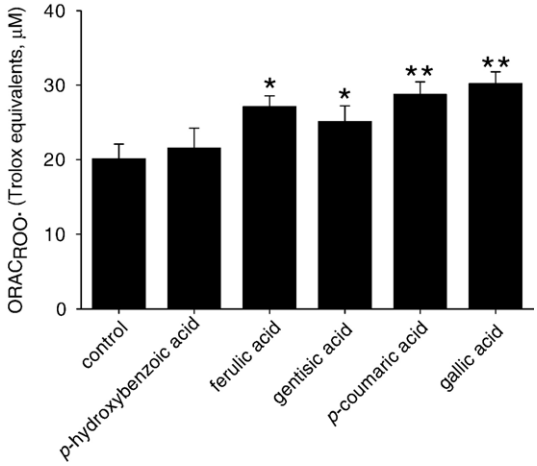

Fig. 3. Effect of phenolic acid administration on total plasma antioxidant capacity assayed as oxygen radical absorbed capacity (ORAC$_{ROO\cdot}$). Plasma antioxidant capacity was analyzed using the ORAC$_{ROO\cdot}$ method. The results represent the mean±S.E.M. of values obtained from six animals in each group. $*P<.05$ and $**P<.01$, significantly different from the control value.

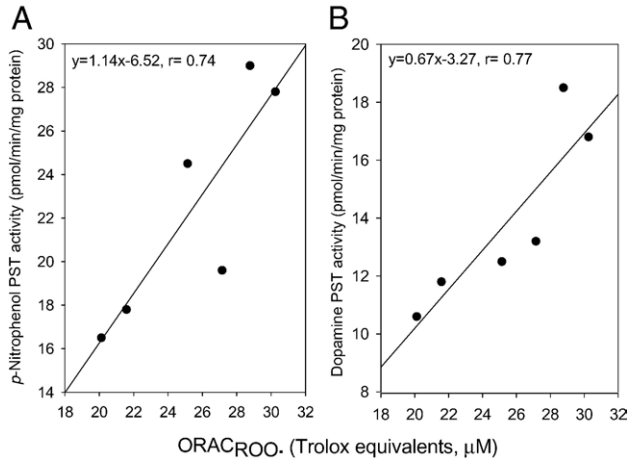

Fig. 4. Effect of phenolic acids on plasma ORAC$_{ROO\cdot}$ values (*x*-axis) (Trolox equivalents, μM) in relation to their (A) *p*-nitrophenol and (B) dopamine PST activity (*y*-axis) (pmol/min per milligram of protein). Each value represents the mean±S.E.M. of six rats.

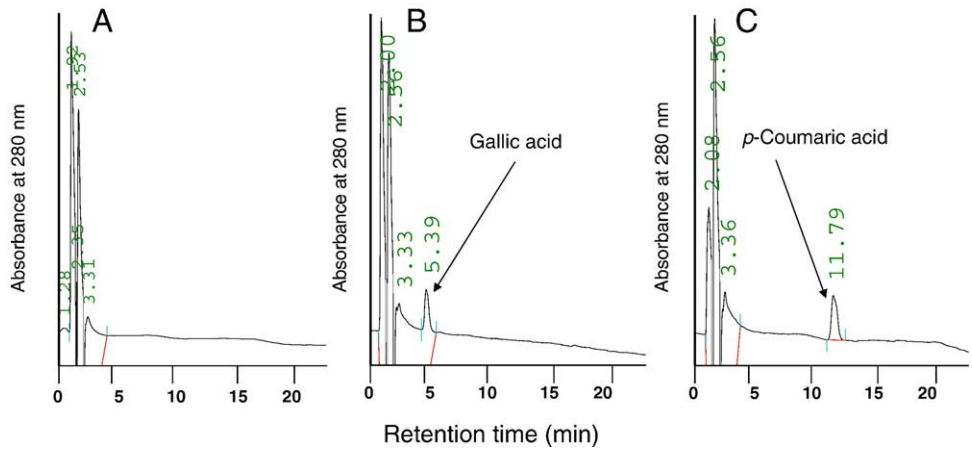

Fig. 5. Typical HPLC chromatograms of rat plasma extracts of blood taken before phenolic acid administration (A), 2.0 h after oral administration of 100 mg/kg gallic acid (B), and 2.0 h after oral administration of 100 mg/kg *p*-coumaric acid (C).

mRNA content by *p*-hydroxybenzoic acid was less than that of ferulic acid. Increase of PST-M mRNA level was also noted in rats treated with ferulic acid, gentisic acid, *p*-coumaric acid and gallic acid as compared with those of control rats. These results are in agreement with the results of corresponding activity of enzymes.

3.4. Effects of phenolic acids on plasma total antioxidant capacity

The $ORAC_{ROO\cdot}$ values in the plasma of rats treated with phenolic acids are shown in Fig. 3. Gallic acid and *p*-coumaric acid supplementation significantly ($P<.01$) elevated the $ORAC_{ROO\cdot}$ values in the plasma as compared to the control group. Average values for $ORAC_{ROO\cdot}$ of *p*-hydroxybenzoic acid, ferulic acid, gentisic acid, *p*-coumaric acid and gallic acid were 21.5±2.6, 27.1±1.4, 25.1±2.1, 28.7±1.7 and 30.3±1.5 μM Trolox equivalent, respectively. The respective $ORAC_{ROO\cdot}$ values of plasma increased 7.5%, 35.5%, 25.5%, 43.5% and 48.5% compared to the control group ($P<.05$). The influence of phenolic acids on PST activity in relation to their plasma $ORAC_{ROO\cdot}$ values is presented in Fig. 4. It was found that there is a significant linear correlation between the influence of phenolic acids on PST-P activity and their $ORAC_{ROO\cdot}$ values. A correlation coefficient ($r=.74$, $P<.05$) was observed between the influence of phenolic acids on PST-P activity and their $ORAC_{ROO\cdot}$ values (Fig. 4A). A similar result can be seen in Fig. 4B, where there is a significant linear correlation between the influence of phenolic acid on PST-M activity and their $ORAC_{ROO\cdot}$ value ($r=.77$, $P<.05$). These results suggested the effects of phenolic acids administered on the activity of PST-P and PST-M activity were well correlated to their $ORAC_{ROO\cdot}$ values.

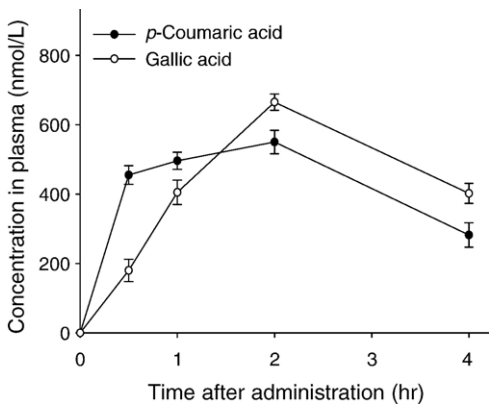

Fig. 6. Time course of changes in concentration of gallic acid and *p*-coumaric acid in rat plasma after oral administration of a single dose of phenolic acid: gallic acid (•) and *p*-coumaric acid (◦). Values are the mean±S.E.M. for three rats.

3.5. HPLC analysis of plasma metabolites in phenolic acid-administered rats

After oral administration of gallic acid or *p*-coumaric acid, each at the same dosage (100 mg/kg of body weight), the plasma concentrations were measured by HPLC. Fig. 5 shows typical HPLC profiles of rat plasma 2.0 h after administration of the phenolic acids. Gallic acid and *p*-coumaric acid were detected in the plasma as the intact forms. No other peaks were detected in the analysis of rat plasma after administration of gallic acid or *p*-coumaric acid when the absorbance of the eluate was monitored at 280 nm (Fig. 5B and C). Fig. 6 shows the time course of changes in the concentrations of gallic acid and *p*-coumaric acid in rat plasma after the administration of these phenolic acids. None of these phenolic acids were detected in plasma before administration. Following oral administration of gallic acid and *p*-coumaric acid, there was a rapid increase in the plasma concentration to 180±32 and 455±26 nmol/L, respectively, at 0.5 h postadministration. The plasma concentration reached a maximum of 665±23 nmol/L at

2.0 h postadministration in the case of gallic acid and 550±33 nmol/L at 2.0 h postadministration in the case of *p*-coumaric acid and then decreased.

4. Discussion

The induction of detoxification enzymes represents a significant mechanism of cancer prevention through which various natural or synthetic chemoprotective agents can act [14]. Several studies have shown that phenolic compounds are able to induce phase II enzymes [15]. In our previous study, it was shown that the *p*-hydroxybenzoic acid, gentisic acid, ferulic acid, gallic acid and *p*-coumaric acid can increase the activities of both PST-P and PST-M. These phenolic acids were also found to possess antioxidant capacity in the $ORAC_{ROO\cdot}$ and TEAC assays, suggesting that phenolic acids may alter sulfate conjugation [11,12]. To clarify the biological action of phenolic acids, in vivo sulfoconjugation experiment was carried out. According to the reports of Krajka-Kuzniak et al. [16] and our preliminary test, we used 14 days for pretreatment. Our experimental results show that ferulic acid, gentisic acid, *p*-coumaric acid and gallic acid can increase both forms of PST activities and mRNA expression in rat liver (Figs. 1 and 2). This induction was seen at the gene expression level. This finding agrees with our previous report for phenolic acid induction of human PSTs [11], which suggested that the liver is an important tissue for toxicant detoxification.

Phenolic acids, especially hydroxycinnamic acids and hydroxybenzoic acid, are secondary plant products, commonly found in plant-derived food stuffs. Ferulic acid and *p*-coumaric acid have been reported to act as scavengers of thiol free radicals [17]. *p*-Coumaric acid also possesses potent antioxidant activity in enhancing the resistance of low-density lipoprotein to oxidation [18]. In addition, *p*-coumaric acid (50 mg/kg body weight) significantly decreased the basal level of oxidative damage in rat colonic mucosa [19], whereas gentisic acid has been reported to have an inhibitory action in the myeloperoxidase system and was able to impair tyrosyl radical catalyzed low-density lipoprotein peroxidation [20]. In addition, gallic acid is a potent natural antioxidant, exhibiting antimutagenic and anticarcinogenic activity, and is expected to reduce the risk of disease and bring health benefits through daily intake [21]. The $ORAC_{ROO\cdot}$ assay is one of the methods used to evaluate the antioxidant biology substrates, ranging from pure compounds such as melatonin and flavonoids to complex matrices such as vegetables and animal tissues [22]. However, there is little information on the in vivo antioxidant activity of phenolic acids. Here, we also examined $ORAC_{ROO\cdot}$ values of plasma in rat treated with phenolic acids that increased the activity of PSTs by oral administration. Our results clearly demonstrated for the first time that orally administered phenolic acids do significantly increase the $ORAC_{ROO\cdot}$ values in rat plasma (Fig. 3). In general, phenolic acids that induced both forms of PST activities were found to have higher $ORAC_{ROO\cdot}$ values. There was a significant correlation between the influence of phenolic acids on both forms of PST activities and their $ORAC_{ROO\cdot}$ values ($r=.74$, $P<.05$; $r=.77$, $P<.05$) (Fig. 4A and B).

In vivo studies showed that the antioxidant potential in humans responds to the oral ingestion of polyphenols [23]. Thus, some substances that exist in the gut are absorbed and may be responsible for the in vivo antioxidant properties of red wine. Piskula and Terao [24] 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 [25]. In order to establish whether phenolic acids make a contribution in the induction of both forms of PST activities and antioxidant activity in vivo, an additional study was conducted by HPLC with diode-array detection. Two phenolic acids, gallic acid and *p*-coumaric acid, were orally administered to rats at the same dosage (100 mg/kg of body weight), and these were found to be absorbed and distributed to the blood as the intact form (Fig. 5). Comparison of the time course of changes in plasma concentrations of the two components showed that gallic acid and *p*-coumaric acid 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 (Fig. 6). This shows a close agreement with prior work, done by Konishi et al. [26], showing that the phenolic acids, especially gallic acid and *p*-coumaric acid, were directly absorbed and disturbed to the blood. In this study, we have also demonstrated that the absorption efficiency of gallic acid was much higher than that of *p*-coumaric in vivo, indicating differences in the absorption characteristics of these two compounds (Fig. 6). These results are in good agreement with the conclusions of the in vitro study reported previously [26]. It might be possible to ascribe the difference in absorption efficiently to the distinct transport characteristics in vivo as was the case in the in vitro study. Solubility of ingested flavonoids was reported to affect the absorption and excretion significantly. Shimoi et al. [27] reported that αG-rutin (a water-soluble flavonoid) was absorbed more efficiently than either quercetin or rutin because the decrease of precipitation in the alimentary tract raised the amount available for absorption. Our results showed that the antioxidant ability of rat plasma was remarkably increased after oral administration of phenolic acids and their metabolites appeared in plasma. These results mean that phenolic acids such as gallic acid and *p*-coumaric acid are absorbed and their plasma metabolites may act as antioxidants in blood circulation.

Recently, it was reported that *p*-coumaric acid effectively decreased oxidative DNA damage in rat colonic mucosa [28]. This effect might be explained by the increased

expression of glutathione *S*-transferase (GST) M2, an important isoform of GST, which is highly expressed in many tissues and plays a protective role against endogenous oxidative in many tissues [29]. It is interesting to observe in this respect that epigallocatechin gallate, one of the major constituents of green tea polyphenols with interesting chemopreventive activities, specifically induces the GST-M2 isoform [30]. Gallic acid, and its catechin derivatives, has demonstrated excellent chemopreventive effects in many target organs challenged with various carcinogens. A number of studies indicate that gallic acid is a potent inducer of phase II drug metabolism enzymes; this molecular mechanism is thought to involve transcriptional up-regulation of phase II genes [31]. Thus, we can reasonably speculate that the antioxidant ability of metabolites of phenolic acids found in plasma may be related to their PSTs inducing effect in phenolic acid-fed rats. Phenolic acids (caffeic acid, gallic acid and ferulic acid) are absorbed and/or metabolized in humans [32] and rats [33]. Especially high concentrations of phenolic acids are found in coffee, apples, citrus fruits and juices, and the bran of cereal grains [34]. The estimated range of consumption is 25 mg–1 g a day depending on diet (fruit, vegetables, grain, teas and coffees) [35]. Therefore, the supplementation of natural phenolic acids through a balanced diet containing enough fruits and vegetables could be the most effective in inducing of phase II sulfate conjugation enzymes.

In conclusion, our results demonstrate that the phenolic acid administration in rats could significantly induce phase II hepatic PSTs and increase plasma antioxidant capacity. There was a significant correlation between the activity of both forms of PST and the antioxidant capacity of $ORAC_{ROO}$ value by phenolic acids ($r=.74$, $P<.05$ and $r=.77$, $P<.05$). Furthermore, orally administered gallic acid and *p*-coumaric acid are directly absorbed and distributed to the blood in rats. This is the first study to provide evidence of direct absorption of phenolic acid as the intact forms. Since PST is a key enzyme to catalyze the xenobiotics metabolism, the increased activity of PST will therefore promote the efficiency of detoxification. Our results provided better understanding of the effects of phenolic acids on human PST activities, as well as information regarding the intake of phenolic antioxidants for human health. The biological implications of these finding could be important for understanding the antioxidant properties of phenolic acids, which show great potential in the induction of phase II chemopreventive enzymes.

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