Induction of Hepatic Antioxidant Enzymes by Phenolic Acids in Rats Is Accompanied by Increased Levels of Multidrug Resistance–Associated Protein 3 mRNA Expression[1]

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ABSTRACT Phenolic acids are widespread in plant foods; they contain important biological and pharmacological properties, some of which were shown to be effective in preventing cancer. We investigated the modulatory effects of phenolic acids on an antioxidant system in male Sprague-Dawley rats. Rats were orally administrated gentisic acid (GEA), gallic acid (GA), ferulic acid (FA), and *p*-coumaric acid (*p*-CA) at a dosage of 100 mg/kg body weight for 14 consecutive days. At this dose, the activities of hepatic superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were greater after administration of all 4 phenolic acids compared with the control group ($P < 0.05$). The activities of these enzymes in the small intestine of rats were also significantly greater after GA and *p*-CA treatment compared with controls. The changes in hepatic CuZnSOD, GPx, and catalase mRNA levels induced by phenolic acids were similar to those noted in the enzyme activities. Oxidized glutathione levels were lower ($P < 0.05$) in the liver of all phenolic acid–supplemented rats, whereas reduced glutathione was markedly higher than in control rats, especially after administration of GA and *p*-CA. The liver homogenates obtained from rats that had been administered phenolic acids had higher oxygen radical absorbance capacity than those obtained from control rats. Immunoblot analysis revealed an increased total level of Nrf2, a transcription factor governing the antioxidant response element in phenolic acid–supplemented rats. Phenolic acid–mediated antioxidant enzyme expression was accompanied by upregulation of multidrug resistance–associated protein Mrp3. These experiments show that modulation of phase II antioxidant enzymes and oxidative status in the liver by phenolic acids may play an important role in the protection against adverse effects related to mutagenesis and oxidative damage. J. Nutr. 136: 11–15, 2006.

KEY WORDS: • *phenolic acid* • *antioxidant gene expression* • *nuclear factor E2–related factor* • *multidrug resistance–associated protein*

Chemoprevention is defined as the use of nontoxic substances, including those found in many foods, to interfere with the processes of cancer development and carcinogenesis before invasion and metastasis occur. One important process in chemoprevention involves modulation of the activity of the so-called phase II antioxidant enzymes, which convert carcinogens to inactive metabolites that are readily excreted from the body, thus preventing their reactions with DNA (1). The induction of antioxidant enzymes by chemoprotective agents was shown to be an effective strategy for protecting cells against multistage carcinogenesis in experimental animals as well as in clinical trials (2). In an examination of the mechanism of the induction of phase II antioxidant enzymes, it was shown that the basic-region leucine-zipper factor, nuclear factor-E2–related factor (Nrf2),[3] is essential for antioxidant responsive element (ARE)-mediated induction (3,4).

Epidemiologic studies have suggested an association between the consumption of phenolic acid–rich foods or beverages and the prevention of many diseases (5). These phenolic compounds exhibit good antioxidant and chemoprotective properties in vivo (6). Previous studies in our laboratory showed that gentisic acid (GEA), ferulic acid (FA), gallic acid (GA), and *p*-coumaric acid (*p*-CA) modulate phase II sulfate conjugative enzymes (7). However, little information on the *in vivo* effects of these phenolic acids on phase II antioxidant enzymes has been published.

The aim of the present study was to investigate the effect of 4 phenolic acids on the activities of antioxidant enzymes and in particular on the expression of hepatic CuZn superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase in the liver and small intestine of rats. Moreover, we evaluated the effects of each of the phenolic acids on the redox state of

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[3] Abbreviations used: AAPH, 2,2′-azobis(2-amidinopropane) hydrochloride; ARE, antioxidant responsive element; β-PE, β-phycoerythrin; BSA, bovine serum albumin; BW, body weight; FA, ferulic acid; GA, gallic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GEA, gentisic acid; GPx, glutathione peroxidase; GR, glutathione reductase GSH, reduced glutathione; GSSG, oxidized glutathione; Mrp, multidrug resistance–associated protein; Nrf2, nuclear factor E2–related factor 2; ORAC, oxygen radical absorbance capacity; *p*-CA, *p*-coumaric acid; PCA, perchloroacetic acid; PMSF, phenylmethylsulfonyl fluoride; ROS, reactive oxygen species; SOD, superoxide dismutase; TBST, Tris-buffered saline Tween-20.

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glutathione and the oxygen radical absorbance capacity (ORAC) in the liver of rats. A battery of hepatic multidrug resistance–associated proteins (Mrp), such as Mrp2 and Mrp3, which are co-induced with phase II antioxidant enzymes via transcription factor Nrf2 (8), were also examined.

MATERIALS AND METHODS

Materials. GEA (2,5-dihydroxybenzoic acid, 98% purity), FA (*trans*-4-hydroxy-3-methoxycinnamic acid, 99% purity), GA (3,4,5-trihydroxybenzoic acid, 98% purity), *p*-CA (*trans*-4-hydroxycinnamic acid), β-NADPH, perchloroacetic acid (PCA), BHT, glutathione reductase (GR), 2,4-dinitrofluorobenzene, β-phycoerythrin (β-PE), sucrose, and Na_2EDTA were obtained from Sigma Chemical. The TRIzol RNA isolation kit was obtained from Life Technologies; primers for RT-PCR, dNTP, reverse transcriptase, and Taq polymerase were obtained from Gibco BRL. Protein assay reagent was purchased from Bio-Rad Laboratories. All other chemicals used were of the highest pure grade available.

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. The rats had free access to water and a casein-based phenolic acid–free diet. The diet contained casein (200 g/kg), corn oil (50 g/kg), sucrose (500 g/kg), cornstarch (150 g/kg), cellulose (50 g/kg), AIN-76 vitamin mix (10 g/kg), AIN-76 mineral mix (35 g/kg), methionine (3 g/kg), and choline bitartrate (2 g/kg) (Harlan Teklad) (9). Food intake and body weight (BW) were recorded daily. After an adaptation period of 1 wk, the rats were randomly divided into 5 groups ($n = 6$/group). To study the effects of phenolic acids on the induction of phase II antioxidant enzymes in rats, the acids (GEA, FA, GA, and *p*-CA) were given daily by gavage to the rats at a dosage of 100 mg/(kg BW·d) for 14 consecutive days. Before injection, the compound was dissolved in propylene glycol, and then mixed with saline (the concentration of propylene glycol was <1%). The control group was treated with vehicle alone. On d 15, the rats were deprived of food for 16 h and anesthetized with diethyl ether. Livers were collected, washed with sterile, ice-cold NaCl (9 g/L) solution, and kept in a dry ice bath. Intestinal lumens were carefully washed with sterile NaCl (9 g/L) solution. Luminal cavities were opened and fat particles and small blood vessels were removed. The mucosal cells from the small intestine were collected and frozen immediately in a dry ice bath. Samples were stored at −80°C until use (not >1 wk). All experimental procedures involving animals were conducted in accordance with the NIH guidelines. This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the National Chung Hsing University, Taichung, Taiwan.

Cytosol preparations. Liver homogenates were prepared with 50 mmol/L Tris buffer containing 0.25 mol/L of sucrose pH 7.5. Intestinal mucosal homogenates were prepared with the same buffer containing 10 mg/L trypsin inhibitor and 10 mg/L phenylmethylsulfonyl fluoride (PMSF). All homogenates were centrifuged at 100,000 × *g* for 1 h at 4°C. Cytosol aliquots were collected and preserved at −80°C for enzymatic assay and Western blot.

Antioxidant enzymes assays. Total SOD activity was determined by the method developed by Spitz and Oberley (10) with slight modifications. The total SOD activity in each sample was calculated using a concurrently run SOD (Sigma Chemical) standard curve, and expressed as U/mg sample protein. Tissue GPx activity was measured by the method developed by Flohe and Gunzler (11). Catalase activity was measured by the method described by Aebi (12). Cytosolic protein content was quantified by a Bio-Rad protein assay with bovine serum albumin (BSA) as the standard.

GSH analysis. Total glutathione [GSH and oxidized glutathione (GSSG)] was measured according to the modified method developed by Tietze (13). The change in absorbance was monitored at 410 nm for 5 min, and GSH and GSSG levels were calculated using pure GSH and GSSG as standards.

Oxygen radical absorbance capacity (ORAC). The ORAC assay was based on the procedure described by Cao and Prior (14). Free radicals were produced by 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), and the oxidation of the fluorescent indicator protein β-PE was measured. Both reagents were prepared in 75 mmol/L phosphate buffer (pH 7.0), and 50 μmol/L Trolox was used as the standard. The liver samples were homogenized in 4 volumes of phosphate buffer in a Thomas homogenizer (20 strokes) and centrifuged at 12,000 × *g* for 10 min at 4°C. The supernatant was deproteinized using 0.25 mol/L PCA and centrifuged at 16,000 × *g* for 15 min. The supernatants were then stored at −80°C before 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 (1:1) sample incubated at 37°C for 15 min. The reaction was initiated by the addition of 20 μL of AAPH (240 mmol/L), and the fluorescence (emission 590 nm, excitation 530 nm) was recorded every 5 min until the reading had declined to <5% of the initial reading. The ORAC values were calculated and expressed as μmol Trolox equivalents/mg protein.

RNA extraction and RT-PCR. RT-PCR was performed to determine the level of antioxidant enzyme gene expression. Total RNA from rat liver tissues was isolated using the TRIzol RNA isolation kit (Life Technologies) as described in manufacturer's manual. cDNA was synthesized with random primers using the Reverse Transcription system (Promega) according to the manufacturer's instructions. Cu/Zn SOD was amplified at 94°C for 45 s, 56°C for 30 s, and 72°C for 45 s for a total of 23 cycles followed by a 10-min extension at 72°C using the following primers: 5′-TCT AAG AAA CAT GGC GGT CC-3′ and 5′-CAG TTA GCA GGC CAG CAG AT-3′. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 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′-CCA TCA CCA TCT TCC AGG AG-3′ and 5′-CCT GCT TCA CCA CCT TCT TG-3′. GPx and catalase were 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: GPx, 5′-CTC TCC GCG GTG GCA CAG T-3′ and 5′-CCA CCA CCG GGT CGG ACA TAC-3′; catalase, 5′-GCG AAT GGA GAG GCA GTG TAC-3′ and 5′-GAG TGA CGT TGT CTT CAT TAG CAC TG-3′. Mrp2 and Mrp3 were amplified at 94°C for 45 s, 55°C for 30 s, and 72°C for 1 min for a total of 25 cycles followed by a 10-min extension at 72°C using the following primers: Mrp2, 5′-GGC TGA GTG CTT GGA C-3′ and 5′-CTT CTG ACG TCA TCC TCA C-3′; Mrp3, 5′-CCG CCT TGC TGC TGG TTA TT-3′ and 5′-CCG ACC TTC TCT CCG CTC TT-3′. 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 UV transillumination. The PCR bands on the photograph of the gel were scanned for quantification using a densitometer linked to a computer analysis system. Net band intensity (background-subtracted intensity) was normalized to GAPDH values and plotted as arbitrary units. Water samples and RNA samples containing no RT were amplified in parallel to ensure that no contaminating DNA was present during PCR.

Western blotting. The cytosolic fraction (supernatant) proteins from liver were measured using a Bio-Rad protein assay with BSA as the standard. Electrophoresis was carried out using SDS-PAGE. After electrophoresis, proteins on the gel were electrotransferred onto an immobile membrane (PVDF; Millipore) with transfer buffer composed of 25 mmol/L Tris-HCl (pH 8.9), 192 mmol/L glycine, and 20% methanol. The membrane was then washed with Tris-buffered saline (10 mmol/L Tris, 150 mmol/L NaCl) containing 0.05% Tween 20 (TBST) and blocked in TBST containing 5% nonfat dried milk. The membrane was further incubated overnight at 4°C with specific antibodies such as Nrf2 (1:2000) and β-actin (1:5000). After hybridization with primary antibodies, the membrane was washed 3 times with TBST, incubated with horseradish peroxidase–labeled secondary antibody for 45 min at room temperature, and then washed 3 times with TBST. Final detection was performed with enhanced chemiluminescence Western blotting reagents (Amersham Pharmacia Biotech).

Statistical Analysis. Results are expressed as means ± SD. ANOVA was used to evaluate differences between multiple groups, and comparisons between the means of treated groups and the control group were made using Dunnett's test. Differences were considered significant at $P < 0.05$.

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RESULTS

After 2 wk of treatment, the growth rate of the phenolic acid–supplemented rats did not differ from that of the control group (data not shown). Rats administered 100 mg/kg BW of FA, GEA, GA, and *p*-CA had significantly($P < 0.05$) elevated hepatic SOD, GPx, and catalase activities compared with the control group. Rats administered FA, GEA, GA, and *p*-CA also had higher intestinal catalase activity than the control group (**Table 1**).

Oxidative stress in tissues generally involves the GSH system; therefore, the level of GSH and the ratio of GSH:GSSG in each group were measured. Due to the changes in the levels of GSH and GSSG, the GSH:GSSG ratio was greater ($P < 0.05$) in the liver of rats administered GA and *p*-CA. The ORAC values in the liver of rats were greater ($P < 0.05$) after treatment with GA and *p*-CA but not with FA and GEA (**Table 2**).

CuZnSOD, GPx, and catalase mRNA expression in liver tissues were modulated simultaneously by phenolic acids. As quantitated by densitometry, the inductions of CuZnSOD by GA, *p*-CA, FA, and GEA were 80.9, 70.4, 68.8, and 8.5%, respectively, of those of the control. GPx and catalase mRNA levels were also higher in rats supplemented with GA, *p*-CA, and FA. These results were supported by the activities of the corresponding enzymes (**Fig. 1**).

As a DNA-binding protein that recognizes the ARE enhancer sequence, Nrf2 functions as an important mediator in the expression of several phase II enzyme genes. The antioxidant enzyme gene contains an ARE sequence similar to that found in the rat phase II genes NAD(P)H:quinine oxidoreductase and glutathione S-transferase A1. To examine further whether Nrf2 expression was modulated by phenolic acids, an immunoblotting analysis was performed (**Fig. 2**). The total levels of Nrf2 protein expression in liver tissues were modulated simultaneously by phenolic acid treatment. These observations were consistent with antioxidant gene expression (Fig. 1), suggesting that an accumulation of Nrf2 protein might contribute to the induction of ARE-mediated antioxidant gene expression after phenolic acid treatment.

Finally, Mrp3 gene expression was elevated by all 4 phenolic acid treatments, and GA elicited the strongest inductive effect (**Fig. 3**). In contrast, individual administration of these phenolic acids had no effect on the Mrp2 levels in rat liver. The housekeeping gene, GAPDH, was not affected by the treatments. Therefore, we hypothesize that the induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance–associated protein 3 mRNA expression.

DISCUSSION

Oxidative stress has been implicated in the pathogenesis of atherosclerosis. The role of diet in the prooxidative and antioxidative processes can both increase and decrease oxidative stress in the body (15). Recent evidence showed clearly that certain bioflavonoids have pharmacokinetic properties similar to those of vitamin E. Dietary phenolic acids may also have physiological antioxidant properties, quenching reactive oxygen species (ROS), thereby potentially modifying pathogenic mechanisms relevant to cardiovascular disease (16). We used 14 consecutive days for pretreatment and examined the effect of phenolic acids on the antioxidant defense system in rats according to the reports of Krajka-Kuzniak et al. (17) and our preliminary test.

The present study demonstrates that GEA, GA, FA, and *p*-CA are concomitant inducers of phase II antioxidant enzymes in the liver and small intestine. Our result is in close agreement with that reported by Krajka-Kuzniak and Baer-Dubowska (18), who found that activities of the cytochrome P450 and phase II enzymes were induced by tannic acid in the liver and kidney of rats. Furthermore, the mRNA expression levels of hepatic CuZnSOD, GPx, and catalase in liver tissues of phenolic acid–supplemented rats were higher than the levels in the control group. The fact that the mRNA expressions of CuZnSOD, GPx, and catalase were altered by the phenolic acid supplement indicates that these enzymes are regulated on a transcriptional level.

Antioxidant activity was demonstrated for phenolic acids in various in vitro systems (7). The ORAC assay is one of the methods used to evaluate the antioxidant activity of biological substrates, ranging from pure compounds such as melatonin and flavonoids to complex matrices such as vegetables and animal tissues. Here, we also examined ORAC values in liver of rats treated orally with phenolic acids. We found that phenolic acids increased the activity of antioxidant enzymes. Our results clearly showed for the first time that orally administered phenolic acids ($P < 0.05$) increase the antioxidant defense system in rat liver. In general, phenolic acids that induce antioxidant enzyme activities have higher antioxidant capacities.

GSH plays an important role in hepatic antioxidation and drug metabolism. High intracellular GSH levels reduce damage and promote better survival under conditions of oxidative stress

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TABLE 1

Effect of phenolic acids (100 mg/kg BW) on hepatic and intestinal antioxidant enzyme activities for 2 wk[1]

	Liver			Small intestinal		
Treatment	Total SOD *U/mg protein*	GSH Peroxidase *nmol/(min·mg protein)*	Catalase *μmol/(min·mg protein)*	Total SOD *U/mg protein*	GSH Peroxidase *nmol/(min·mg protein)*	Catalase *μmol/(min·mg protein)*
Control	23.1 ± 4.2	20.1 ± 3.9	10.5 ± 3.4	21.3 ± 2.8	18.8 ± 1.8	6.7 ± 0.4
FA	29.6 ± 2.4*	30.1 ± 2.3*	13.6 ± 1.8*	27.9 ± 2.2*	20.8 ± 2.4	10.1 ± 1.8*
GEA	33.7 ± 3.1*	29.8 ± 1.2*	16.5 ± 3.6*	22.1 ± 2.1	26.4 ± 2.5*	11.4 ± 4.6*
GA	36.4 ± 2.5*	34.1 ± 1.7*	23.7 ± 2.6*	28.1 ± 4.7*	23.7 ± 4.2*	15.3 ± 4.3*
p-CA	40.1 ± 4.6*	34.1 ± 1.6*	19.3 ± 3.3*	27.6 ± 3.3*	30.1 ± 4.6*	15.9 ± 2.3*

[1] Values are means ± SD, $n = 6$. *Different from the control group, $P < 0.05$ (Dunnett's test).

TABLE 2

Effect of phenolic acid (100 mg/kg BW) on GSH, GSSG, the ratio of GSH:GSSG, and oxidative capacity (ORAC) in livers of rat exposed to various phenolic acids for 2wk[1]

Treatment	GSH *μmol/mg protein*	GSSG *μmol/mg protein*	GSH:GSSG ratio	ORAC *μmol Trolox equivalents/mg protein*
Control	10.6 ± 2.9	1.2 ± 0.2	9.3 ± 1.8	7.9 ± 0.8
FA	13.6 ± 5.3	0.8 ± 0.1*	16.6 ± 4.5	10.1 ± 1.2
GEA	15.1 ± 4.5	0.7 ± 0.1*	19.6 ± 4.1	10.7 ± 1.9
GA	18.5 ± 6.3	0.9 ± 0.1*	24.7 ± 7.7*	11.5 ± 1.1*
p-CA	16.4 ± 3.2	0.8 ± 0.2*	22.1 ± 9.8*	11.2 ± 1.8*

[1] Values are means ± SD, $n = 6$. *Different from the control group, $P < 0.05$ (Dunnett's test).

(19). Induction of the hepatic GSH antioxidant system by chemopreventive agents was reported in several studies (20). The result of the present study demonstrated that pretreatment with phenolic acids significantly decreased the level of GSSG in the liver, resulting in an increase in the GSH:GSSG ratio, presumably by stabilizing GSH. Changes in the GSH and GSSG levels significantly increased the GSH:GSSG ratio in the liver of rats fed phenolic acids, whereas it was markedly ($P < 0.05$) increased after GA and *p*-CA treatment, suggesting that phenolic components were very effective at increasing the antioxidant status in the liver.

The transcriptional activation of phase II antioxidant enzymes has been traced to a *cis*-acting transcriptional enhancer called ARE, or alternatively, the electrophile response element. It was shown that the transcription factor Nrf2 positively regulated the ARE-mediated expression of phase II detoxification enzyme genes (21). The most significant finding in our study was the involvement of the Nrf2 pathway in phenolic acid–mediated antioxidant enzyme gene induction, suggesting that increased expression of the Nrf2 protein may play a key role in phenolic acid–induced antioxidant gene activation.

The importance of the multidrug resistance proteins 2 and 3 (Mrp2 and Mrp3) has been recognized during the last few years

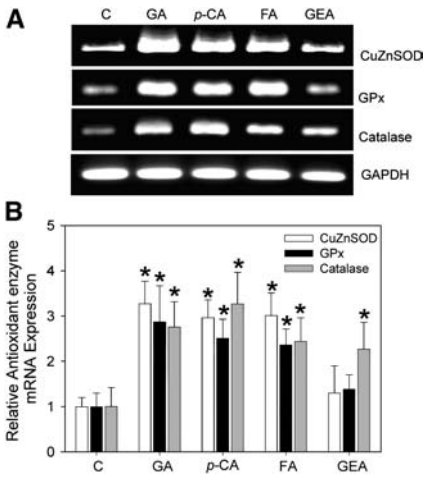

FIGURE 1 CuZnSOD, GPx, and catalase mRNA expression in the livers of control and phenolic acid–supplemented rats. (*A*) Agarose gel (1.8%) electrophoresis of the PCR products. (*B*) The ratios of CuZnSOD, GPx, and catalase to GAPDH mRNA in control and phenolic acid–supplemented rats. Values are means ± SD, $n = 3$. Asterisks indicate different from the control group *$P < 0.05$; **$P < 0.01$.

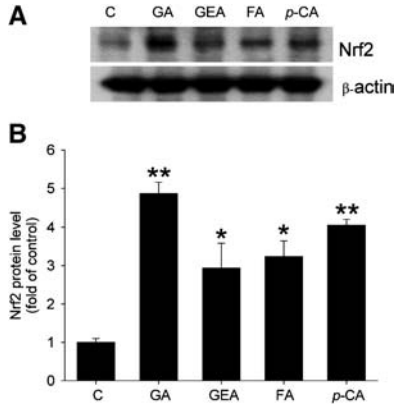

FIGURE 2 Expressions of Nrf2 protein in rat liver. Cytosol proteins (50 μg) were electrophoresed on a 12% polyacrylamide gel. The upper part of the figure indicates an original blot (*A*); the lower part represents the results of densitometric analyses (*B*). Values are means ± SD, $n = 3$. Asterisks indicate different from the control group *$P < 0.05$; **$P < 0.01$.

because of their function as transporters of organic anions and conjugates and their involvement in hepatic detoxification and tissue-specific distribution of drugs (22). In our study, we also demonstrated that phenolic acids markedly increased hepatic Mrp3 mRNA levels but left Mrp2 mRNA levels unchanged. Mrp3 was shown to be induced by activators of the constitutive androstane receptor and an antioxidant/electrophile responsive element (23). The consensus ARE sequences such as 5′-ttgagttagct-3′ and 5′-ttgagacagca-3′ are located between nt −1240 and −1229, and between nt −2020 and −2010 of the rat Mrp gene, respectively, suggesting that increased expression of Nrf2 protein may be related to phenolic acid–induced Mrp gene activation.

It was reported recently that *p*-CA acid effectively decreased oxidative DNA damage in rat colonic mucosa (24). A number of studies indicated that GA is a potent inducer of phase II drug metabolism enzymes; the molecular mechanism may involve transcriptional upregulation of phase II genes (25). Thus, we can reasonably speculate that increasing the hepatic antioxidant capacity may be related to the inducing effect of anti-

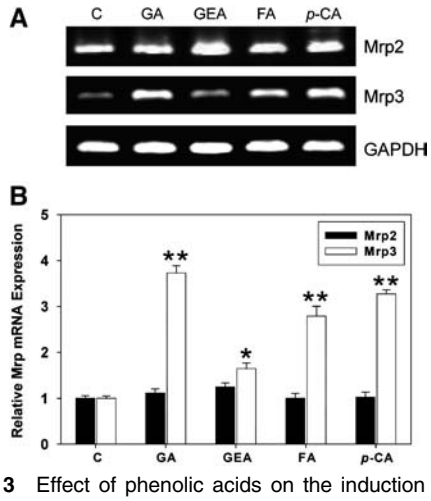

FIGURE 3 Effect of phenolic acids on the induction of Mrp2 and Mrp3 mRNA expression in rat liver. The upper part of the figure indicates an original blot (*A*); the lower part represents the results of densitometric analyses (*B*). Induction folds of Mrp2 and Mrp3 were quantitated by densitometry based on the relative amount of control. Values are means ± SD, $n = 3$. Asterisks indicate different from the control group *$P < 0.05$; **$P < 0.01$.

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oxidant enzymes in phenolic acid–supplemented rats. Phenolic acids are absorbed and/or metabolized in humans and rats (6). Especially high concentrations of phenolic acids are found in coffee, apples, citrus fruits and juices, and the bran of cereal grains. Estimated consumption ranges from 25 mg to 1 g/d depending on diet (fruit, vegetables, grain, teas, and coffees) (26). Therefore, the supplementation of natural phenolic acids through a balanced diet containing enough fruits and vegetables could be the most effective way in which to induce phase II antioxidant enzymes.

In conclusion, our results indicate that phenolic acids significantly induce phase II hepatic antioxidant enzyme and increase the antioxidant status of liver. These phenolic acids seem to selectively induce hepatic mRNA transcripts for CuZnSOD, GPx, and catalase, likely through upregulation of gene transcription as well as the Nrf2 transcription factor. Furthermore, orally administered GA and *p*-CA markedly increased the expression of the conjugate export pump Mrp3 in rat liver. Such a regulation may involve, at least in part, increased Nrf2 induction in rat liver and may contribute to the chemoprevention properties of phenolic acids. CuZnSOD, GPx, and catalase were the key enzymes to catalyze the metabolism of xenobiotics; therefore, the increased activity of these antioxidant enzymes will promote the efficiency of detoxification. Our results provide a better understanding of the effects of phenolic acids on antioxidant enzyme activities in rats as well as information regarding the intake of phenolic antioxidants for human health. The biological implications of these findings 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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