Univ. Bull. Chiba Inst. Sci. 13. 11–20. 2020
【Original Article】

Zerumbone Elevates Phase II, but not Phase I, Xenobiotic-Metabolizing Enzymes in Mouse Liver

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In order to characterize the biological actions of zerumbone (ZER), a cancer-suppressive component from rhizomes of *Zingiber zerumbet* Smith, its effects on the activities of various xenobiotic-metabolizing enzymes were analyzed. Mice were treated orally with ZER (10-100 mg/kg, once a day for 4 successive days) and then the enzyme activities in mouse organs were examined. Within the liver ZER increased glutathione S-transferase (GST) and quinone reductase (QR) activities in a dose-dependent manner up to 204% and 168% of the control, respectively. As the GST activation was most apparent with 1,2-dichloro-4-nitrobenzene as substrate, ZER seems to mainly induce the activity of class μ GST, which was supported by the result of a Western blotting experiment. In addition, contents of glutathione (reduced form) were slightly higher in the ZER-treated liver. Similarly, small intestine GST and QR activities elevated to 861% and 256% of the control, respectively, upon ZER treatment. In contrast, the activities of those enzymes fluctuated little in kidney, lung or brain of ZER-treated mice. As for the phase I enzymes in the liver including cytochrome P450 and cytochrome b_5; activities of 7-ethoxycoumarin *O*-deethylase, 7-methoxyresorufin *O*-demethylase, 7-ethoxyresorufin *O*-deethylase and 7-pentoxyresorufin *O*-dependentylase , ZER caused only marginal changes. Our study clearly indicated that ZER selectively activates phase II enzymes in the liver and small intestine, and thus can be considered to be a promising nutraceutical for cancer chemoprevention.

INTRODUCTION

Cancer chemoprevention, the concept proposed by Sporn (1976),[1)] is the prevention of cancer by means of chemicals through direct treatment or dietary supplement. As to the biological activities of antitumor chemicals, either synthetic or naturally occurring, they have been strongly suggested to act through modulations of drug-metabolizing enzymes by affecting their expression or activation.[2,3)] Many carcinogenic and mutagenic substances (procarcinogens) do not possess strong cell-damaging activity by themselves, but they are amenable to conversion by phase I xenobiotic-metabolizing enzymes (e.g., cytochrome P450) into active chemicals such as electrophilic derivatives (electrophiles), which may exert tumor induction or other adverse effects on a cell (Fig.1). Importantly, another enzyme group, termed as “phase II” enzymes [e.g., glutathione S-transferase (GST) and quinone reductase (QR)], is capable of metabolizing and detoxifying those bioactive intermediate compounds. Thus, it is tempting to hypothesize that the balance between the phase I and phase II enzyme activities may be the most important factor in deciding whether a chemical would induce or suppress cancer in a living organism. Therefore, an intensive analysis of the changes in the activities of both enzyme types is important and useful to define the mechanisms for the cancer chemoprevention.

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(Received September30 2019; Accept December 9 2019)

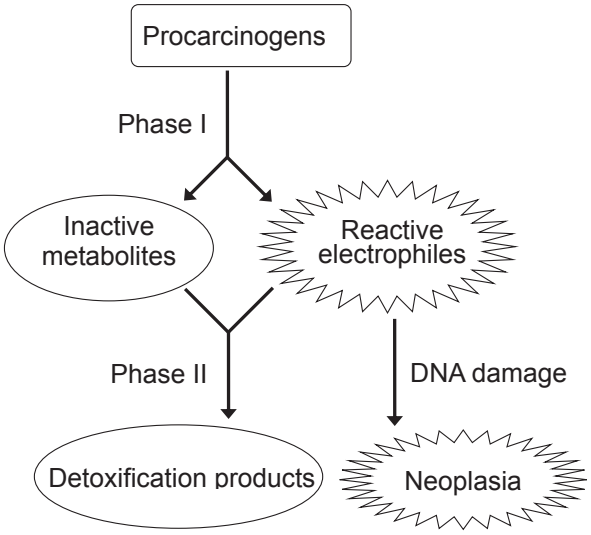

Fig. 1. Roles of phase I and phase II enzymes in the metabolism of carcinogenic substances.

The plants belonging to Zingiberaceae family have been cultured since ancient times mainly in Southeast Asia and widely used as spice and traditional folk medicine.[4] For instance, a wild zinger *Zingiber zerumbet* Smith is employed for its anti-inflammatory effects. Chemical analyses of the essential oil of its rhizomes have resulted in identification of a sesquiterpene compound zerumbone (ZER; 2,6,9,9-tetramethyl-[2*E*,6*E*,10*E*]-cycloundeca-2,6,10-triene-1-one) as one of the most active components in the plant (Fig. 2). ZER has been found to exert a wide variety of biological activities against inflammation,[5] tumors,[6] aberrant crypt foci,[7,8] Epstein-Barr virus,[9] cyclooxygenase-2[5] and tumor necrosis factor.[10]

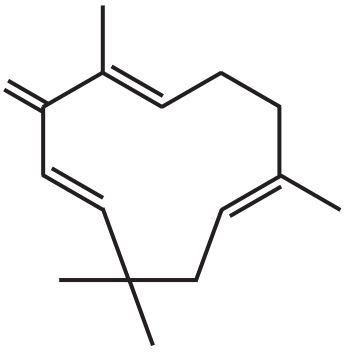

Fig. 2. Chemical structure of ZER

In relation to the cancer chemoprevention, ZER has been found to elevate the activities of phase II enzymes in RL34 cells [11]. Little, however, is known about the ZER action on phase I enzymes. As the balance between the phase I and phase II enzyme activities is considered to be a critical factor, its determination would give us important information to understand the actions of ZER on damaging chemicals and to clarify the detoxifying mechanisms. The idea prompted us to examine the manner how ZER affects those enzymes.

The present study represents a first detailed analysis on ZER-induced modulation of xenobiotic metabolizing enzymes. Here, we showed that ZER enhances phase II enzymes but causes little change on phase I enzymes. The findings strongly suggest that ZER exerts cancer chemoprevention mainly by affecting phase II enzymes. Importantly, ZER is active on the enzymes in mouse liver and small intestine, but not in kidney, lung or brain. As its biological effects are limited to detoxification of ingested substances, ZER can be considered to be a promising chemopreventive agent.

MATERIALS AND METHODS

Materials

ZER was isolated in 99% purity from the zinger rhizomes of *Zingiber zerumbet* as previously reported.[9] The purity and identification were checked by means of HPLC and spectra analyses.[4] Chemicals and reagents were of analytical grade and obtained commercially from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise stated.

Animal care and treatment

Four-week-old mice (ddY strain, male) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in the Health Sciences University of Hokkaido. Mice were maintained under a controlled environment (23 ± 1˚C with constant humidity of 55 ± 5% and a 12 h light/dark cycle) and provided with water and diet *ad lib*. Chemical samples were administered to mice orally once a day as a 2% gum Arabic suspension. Animals received either a vehicle (control group) or ZER (experimental group; 10, 25, 50 and 100 mg/kg/day) orally for the period of 4 days. The doses used in the present study were chosen based on the inhibitory effect of ZER on azoxmethane-induced colonic aberrant crypt foci.[7] In order to validate the assay protocols employed in the present study, mice were treated, in parallel, with 100 mg/kg/day of positive control chemi-

cals, 3-*tert*-butyl-4-hydroxyanisole (BHA; Kanto Kagaku Co., Tokyo, Japan)[12,13] and phenobarbital (PB)[14] orally for the period of 4 days. For analysis of glutathione contents, mice were fed with a powdered diet supplemented with 1% BHA (w/w) for 7 days. Twenty-four hours after the last treatment the mice were sacrificed by decapitation. Tissues were removed, washed with ice-cold 1.15% potassium chloride and blotted briefly.

They were then weighed and subjected to the preparation of cytosolic and microsomal fractions as essentially described before.[15] Briefly, livers, kidneys, lungs and brains were homogenized with Potter-Elvehjem homogenizers in 5 volumes, 6 volumes, 12 volumes and 20 volumes of ice-cold 1.15% potassium chloride, respectively. In the case of small intestine, the mucosa was obtained by mechanical scraping and the samples from 3 mice were combined in total of 10 mL of 1.15% potassium chloride, which were then homogenized. The homogenates were first centrifuged at 9,000 x g for 20 minutes and the resultant supernatants were centrifuged at 105,000 x g for 60 minutes at 4˚C. The supernatants and pellets from the second centrifugation were referred to as cytosolic and microsomal fractions, respectively, and stored at -80˚C until use. Protein contents in the samples were determined by the Lowry-Folin method.[16]

Measurement of enzyme activities

Cytosolic GST activity with 1-chloro-2,4-dinitrobenzene (CDNB; Kanto Kagaku, Tokyo, Japan) or 1,2-dichloro-4-nitrobenzene (DCNB; Kanto Kagaku, Tokyo, Japan) as a substrate were determined by the method of Habig *et al*.[17] Similarly, cytosolic GST activity with 4-nitroquinoline 1-oxide (4NQO; Sigma, MO, USA) as a substrate was determined according to Stanley *et al*.[18] Cytosolic QR activity with menadione (Sigma, MO, USA) as a substrate was determined by the method of Ernster.[19]

Microsomal 7-ethoxycoumarin *O*-deethylase (ECOD) activity was determined by the method of Matsubara *et al*.[20] Microsomal activities of 7-methoxyresorufin *O*-demethylase (MROD), 7-ethoxyresorufin *O*-deethylase (EROD) and 7-pentoxyresorufin *O*-depentylase (PROD) were determined by measuring the fluorescence of resorufin.[21,22]

Measurement of glutathione contents

Contents of glutathione species in mouse livers were determined by enzymatic methods.[23,24] Briefly, livers were perfused with ice-cold 1.15% potassium chloride, homogenized and immediately precipitated with 25% metaphosphoric acid. After centrifugation, the resultant supernatant was subjected to determination of the whole GSH content. Oxidized glutathione (GSSG) was analyzed similarly except for preincubating the above supernatant with 2-vinylpyridine and triethanolamine for 1 h in the dark. The amount of reduced glutathione (GSH) was obtained as the difference between the whole glutathione and GSSG.

Western blot analysis of GST isozymes

Western blot analysis was performed as described previously.[15] Briefly, the cytosolic fraction from the liver was dissolved, blotted and immunoreacted by using polyclonal rabbit antiserum against GST α (PCF 403), μ (PCF408) and π (PCF401) from YLEM (Rome, Italy). Signals were quantified by densitometry with a densitograph (Lumino CCD Model AE-6930, ATTO, Tokyo, Japan).

Measurement of cytochrome P450 and cytochrome b_5

Contents of the cytochromes in the liver were determined according to Omura and Sato.[25] Briefly, CYP was determined by measuring the difference in absorbance between 450 and 490 nm and using an absorption coefficient of 91 $mM^{-1}cm^{-1}$. Cytochrome b_5 was determined by measuring the difference in absorbance between 409 and 424 nm with an absorption coefficient of 185 $mM^{-1}cm^{-1}$.

Statistical analysis

Statistical analyses were performed by Dunnett test. Differences with P values < 0.05 were considered statistically significant.

RESULTS

Effects of ZER on body and organ weights

In the mice orally administered with ZER for 4 days, no abnormality was detected by macroscopic observation of the organs. At the dose of 100 mg/kg/day, there was no significant change in the weights of whole body between control and ZER-treated mice (control, 31.7 ± 1.7; ZER, 32.4 ± 0.9 g) or any organs examined [kidney: control, 0.49 ± 0.05; ZER, 0.54 ± 0.03 g; small intestine: control, 1.74 ± 0.21; ZER, 1.76 ± 0.13 g; lung: control, 0.26 ± 0.03; ZER, 0.24 ± 0.05 g; brain: control, 0.47 ± 0.03; ZER (50 mg/kg/day), 0.47 ± 0.04 g]. Only the liver weights increased slightly at 100 mg/kg/day (control, 1.60 ± 0.22; ZER, 2.00 ± 0.16 g, $p < 0.01$). Similarly,

treatments with control chemicals, BHA and phenobarbital, also induced a slight increase in the liver weight by 12% and 37%, respectively (data not shown).

Effects of ZER on the activities of GST in the liver

A preliminary experiment showed that ZER significantly increased the GST activities in the liver; thus, we investigated the changes in detail. GST comprises several isozymes with varying characteristics and mouse liver is known to possess α, μ and π classes as main isozymes.[26] Here we analyzed the enzyme activities by using different substrates, CDNB (for the whole isozymes), DCNB (mainly for μ class) and 4NQO (for μ and π classes).[26,27]

As shown in Fig. 3, ZER-dependent increase in the GST activity was observed with every substrate: The extents of elevation, however, were greater with DCNB and 4NQO than CDNB (for instance, an increase by 94% and 104% vs. 65% at 100 mg/kg/day). This suggested major contribution of μ class GST therein. Then, we estimated relative amounts of those isozymes by Western blotting analysis: Class μ was the main GST isozyme that increased in the protein amount upon treatment of 50 mg/kg ZER (α class: control, 1.00 ± 0.14, ZER, 1.13 ± 0.20; μ lass: control, 1.00 ± 0.19, ZER, 1.33 ± 0.28, $p < 0.05$; class1 π : control, 1.00 ± 0.15, ZER, 1.04 ± 0.12).

These changes were comparable to those upon treat-

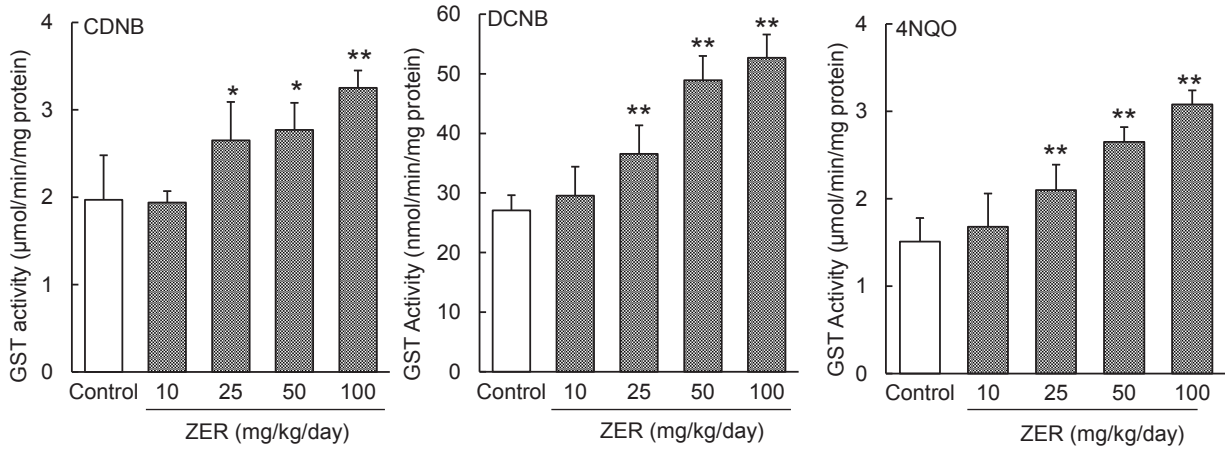

Fig. 3. Effects of ZER on mouse liver GST activities. Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of three to six animals. Significant differences are indicated as follows: *$P < 0.05$ and **$P < 0.01$ compared with control. CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; 4NQO, 4-nitroquinoline 1-oxide.

Table 1 Effects of ZER on mouse liver glutathione contents

ZER (mg/kg/day)	Total glutathione (nmol/mg protein)	GSH content (nmol/mg protein)	GSSG content (nmol/mg protein)
0	3.96 ± 0.30 (100)	3.91 ± 0.30 (100)	0.0235 ± 0.0043 (100)
25	3.91 ± 0.81 (99)	3.87 ± 0.80 (99)	0.0223 ± 0.0029 (95)
50	4.47 ± 0.62 (113)	4.42 ± 0.61 (113)	0.0243 ± 0.0066 (103)
100	4.97 ± 0.65 (126)*	4.91 ± 0.64 (126)*	0.0268 ± 0.0047 (114)

Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of six animals (percentage of the control). Significant differences are indicated as follows: *$P < 0.05$ compared with control. GSH, reduced glutathione; GSSG, oxidized glutathione.

ment with the positive control, BHA: GST activities with CDNB, DCNB and 4NQO elevated by 121%, 170% and 179%, respectively (data not shown).

GST is the enzyme which functions for detoxification of a variety of chemicals through conjugation with GSH. As the content of GSH in the liver is also a factor affecting the efficacy of the GST function, we determined its fluctuation (Table 1): A slight elevation (approximately 26%) of the reduced form of GSH was found to accompany the ZER treatment. This increase might contribute to enhance the detoxifying capacity of the GST enzymes. As for GSSG (the oxidized form) content, ZER caused little difference. A higher increase (44%) in the level of liver GSH was produced by BHA-containing diet (data not shown).

Effects of ZER on the activities of GST in the other organs

Then, we questioned whether the above changes in GST activities occur only in the liver. Fig. 4 and Table 2 show the results from intensive analyses: Briefly, ZER elevated the GST activities greatly in small intestine and slightly in kidney. In contrast, there was no elevation in lung and brain. BHA elevated the GST activity by 40% in small intestine, by 14% in kidney and by 86% in lung for the CDNB substrate (data not shown).

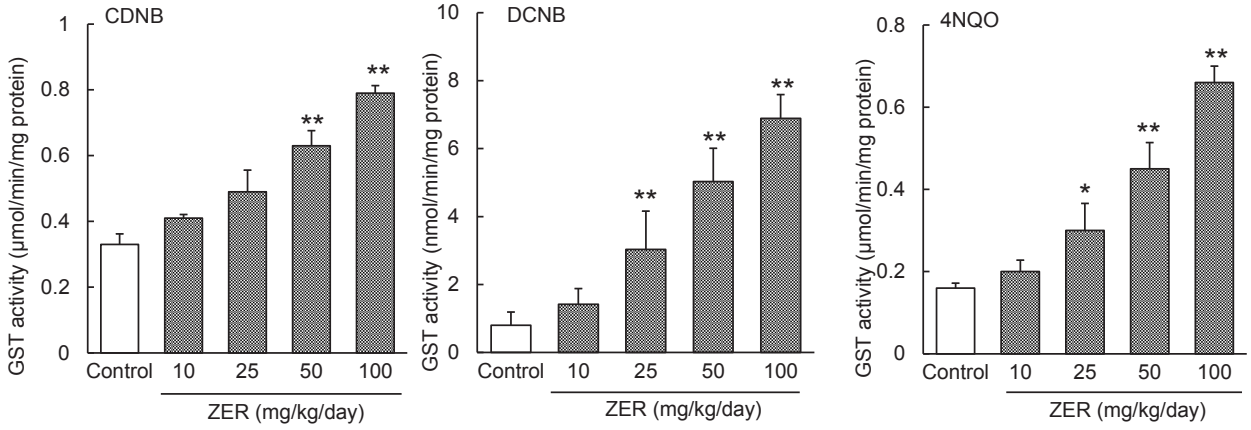

Fig. 4. Effects of ZER on mouse small intestine GST activities. Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of four to six animals. Significant differences are indicated as follows: *$P < 0.05$ and **$P < 0.01$ compared with control. CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; 4NQO, 4-nitroquinoline 1-oxide.

Table 2 Effects of ZER on GST activities in mouse kidney, lung and brain

	Kidney			Lung		Brain
ZER (mg/kg/day)	CDNB (μmol/min/mg protein)	DCNB (mol/min/mg protein)	4NQO (μmol/min/mg protein)	CDNB (μmol/min/mg protein)	4NQO (μmol/min/mg protein)	CDNB (μmol/min/mg protein)
0	0.404 ± 0.032 (100)	8.05 ± 1.42 (100)	0.293 ± 0.010 (100)	0.293 ± 0.066 (100)	0.166 ± 0.032 (100)	0.259 ± 0.049 (100)
10	0.416 ± 0.026 (103)	7.79 ± 1.46 (97)	0.320 ± 0.023 (109)	NT	NT	0.268 ± 0.041 (103)
25	0.415 ± 0.040 (103)	6.98 ± 1.01 (87)	0.297 ± 0.037 (101)	0.274 ± 0.029 (94)	0.166 ± 0.025 (100)	0.256 ± 0.032 (99)
50	0.479 ± 0.033 (119)**	9.23 ± 1.04 (115)	0.338 ± 0.034 (115)	0.300 ± 0.047 (102)	0.187 ± 0.030 (113)	0.269 ± 0.037 (104)
100	0.499 ± 0.044 (124)**	9.07 ± 0.89 (113)	0.324 ± 0.025 (111)	0.314 ± 0.050 (107)	0.193 ± 0.038 (116)	NT

Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of five to six animals (percentage of the control). Significant differences are indicated as follows: **$P < .01$ compared with control. NT, not tested.

Effects of ZER on the activities of QR

We analyzed ZER effects on QR, another major phase II enzyme (Table 3). Basically, the results paralleled to those for GST: Substantial elevation in liver and small intestine, slight elevation in kidney, and no change in lung or brain.

Effects of ZER on the markers for CYP enzymes

Since CYP enzymes account for most of the phase I enzyme activities in the liver,[3] here we focused on the CYP enzymes and determined the contents of CYP and cytochrome b_5, an electron donor for CYP.[25] Obviously, there was no fluctuation of CYP and cytochrome b_5 due to ZER treatment (Table 4).

To further confirm the above point, we analyzed the activities of ECOD, which was used as a marker of a wide range of CYP (for CYP1A1, CYP1A2 and CYP2B).[28] Similarly, we employed MROD as a marker of CYP1A2,[29] EROD as a marker of CYP1A1 and CYP1B1,[30] and PROD as a marker of CYP2B.[31] As shown in Table 4 ZER caused no change in those enzyme activities in mouse liver; the only apparent change was a dose-dependent increase in EROD activity.

Discussion

The present study has demonstrated that oral administration of ZER, a sesquiterpene compound isolated from *Zingiber zerumbet* Smith, enhances the activities of the drug-metabolizing phase II enzymes GST and QR in the mouse liver.

There have been several lines of evidence suggesting that the induction of phase II enzymes such as GST and QR is one of the critical mechanisms for protecting mammals against toxicity of electrophiles, including many carcinogens.[2,3] GSTs are a superfamily of dimeric isozymes that catalyze the conjugation of various toxic electrophilic molecules to glutathione.[26] Major cytosolic GST isozymes are classified into three classes (α , μ and π) on the basis of their structural and immunological characteristics. They have overlapping substrate specific-

Table 3 Effects of ZER on QR activities in mouse organs

ZER (mg/kg/day)	Liver (nmol/min/mg protein)	Kidney (nmol/min/mg protein)	Small intestine (nmol/min/mg protein)	Lung (nmol/min/mg protein)	Brain (nmol/min/mg protein)
0	159.6 ± 22.2 (100)	426.8 ± 25.03 (100)	143.0 ± 15.34 (100)	54.53 ± 5.24 (100)	123.5 ± 19.8 (100)
10	163.7 ± 62.7 (103)	477.0 ± 27.23 (112)*	241.0 ± 10.74 (169)**	NT	122.8 ± 21.2 (99)
25	192.3 ± 43.6 (120)	425.1 ± 37.82 (100)	247.1 ± 11.60 (173)**	54.57 ± 3.06 (100)	112.3 ± 23.1 (91)
50	257.7 ± 32.6 (161)**	508.4 ± 47.14 (119)**	274.9 ± 17.42 (192)**	59.06 ± 11.64 (108)	129.5 ± 34.7 (105)
100	267.7 ± 67.8 (169)**	538.5 ± 10.29 (126)**	365.9 ± 21.04 (256)**	56.54 ± 6.85 (104)	NT

Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of three to six animals (percentage of the control). Significant differences are indicated as follows: *$P < .05$ and **$P < .01$ compared with control. NT, not tested.

Table 4 Effects of ZER on the markers for CYP enzymes

ZER (mg/kg/day)	CYP (nmol/mg protein)	Cytochrome b_5 (nmol/mg protein)	ECOD (nmol/min/mg protein)	MROD (pmol/min/mg protein)	EROD (pmol/min/mg protein)	PROD (pmol/min/mg protein)
0	0.38 ± 0.05 (100)	0.24 ± 0.02 (100)	1.65 ± 0.23 (100)	40.54 ± 4.55 (100)	33.91 ± 8.01 (100)	3.54 ± 0.44 (100)
10	0.35 ± 0.03 (92)	0.20 ± 0.02 (83)	1.56 ± 0.19 (95)	32.41 ± 2.93 (80)	31.15 ± 8.46 (92)	2.61 ± 0.41 (74)
25	0.35 ± 0.06 (92)	0.20 ± 0.04 (83)	1.47 ± 0.25 (89)	37.73 ± 4.91 (93)	34.54 ± 9.13 (102)	3.42 ± 1.19 (97)
50	0.38 ± 0.06 (99)	0.24 ± 0.03 (100)	1.62 ± 0.10 (98)	34.86 ± 5.46 (86)	40.57 ± 7.05 (120)	3.97 ± 1.12 (112)
100	0.37 ± 0.05 (98)	0.26 ± 0.03 (108)	1.75 ± 0.50 (106)	36.53 ± 6.69 (90)	55.00 ± 18.1 (162)**	3.95 ± 0.66 (112)

Mice were treated orally with ZER or vehicle once a day for 4 days. Date are mean ± standard deviation values of four to six animals (percentage of the control). Significant differences are indicated as follows: **$P < .01$ compared with control. CYP, cytochrome P450; ECOD, 7-ethoxycoumarin *O*-deethylase; MROD, 7-methoxyresorufin *O*-deethylase; EROD, 7-ethoxyresorufin *O*-deethylase; PROD, 7-pentoxyresorufin *O*-dependentylase.

ities and physiological functions. Judging from the substrate specificities (Fig. 3) and Western blotting analysis of the GST isozymes, treatment with ZER was found to increase mainly the class μ isozyme. The μ isozyme is known to contribute to detoxification of the carcinogen 4NQO[27] and aflatoxin B_1.[32] It has also been suggested that *trans*-4-hydroxy-2-nonenal, a highly reactive lipid peroxidation product, and *trans*, *trans*-muconaldehyde, a putative myelotoxic metabolite of benzene, are substrates of the μ isozyme.[33] In addition, ZER slightly elevated the liver content of the reduced form of glutathione, the GST substrate (Table 1). This change may also contribute to the augmentation of the phase II enzyme functions.

QR catalyzes the two-electron reduction of quinones and their derivatives to hydroquinone, thereby protecting cells from the damaging electrophilicity of semiquinones and oxygen radical production which results from one-electron reduction of quinines.[34] Since oxidative stress is considered to be closely associated with the carcinogenic process,[35,36] ZER induction of phase II enzymes is likely to be an important contribution to cell protection not only by the conjugative detoxification of the above-mentioned carcinogens but also by scavenging properties against reactive oxygen species.

Herein the actions of ZER on the phase II enzyme activation were apparently tissue-specific, as there was marked elevation of the GST activity due to ZER in the liver and small intestine in contrast to its marginal changes in kidney, lung and brain. There are several other studies reporting such tissue-specific responses to the GST induction: For example, a polyphenolic gallic acid exerts strong GST stimulation in liver and colon,[37] whereas the effects of an isothiocyanate sulforaphane are rather weaker in liver and colon than in forestomach, duodenum and bladder.[38] Those differences may result largely from complex interactions between the chemicals and tissues (or cells).

As for the phase I enzymes, CYP isozymes account for most of their activities in the liver.[3] The present study has shown that ZER does not affect the phase I enzymes. As seen in Table 4, ZER brought about no change in the amount of liver CYP and cytochrome b_5 (an electron donor for CYP). Furthermore, a number of CYP markers examined were affected little by ZER.

With EROD, a CYP1A1 marker, ZER elevated the enzyme activity by 62% at the dose of 100 mg/kg/day. CYP1A1 is the main isozyme involved in conversion of benzo[*a*]pyrene to reactive metabolites; however, recent investigation has suggested that inducible CYP1A1 is important in detoxication and protection against benzo[*a*] pyrene toxicity.[37] Thus, CYP1A1 may be relevant to the ZER chemoprevention. On the other hand, PB, the positive control compound, elevated liver EROD activity (by 245%; data not shown) to much a greater extent than ZER. Together with the total CYP activity and the EROD elevation due to ZER, the present analyses indicate that ZER exerts little, if any, effect on the phase I enzymes.

The Phase I enzyme activities, mainly CYPs, are regulated by several PXR receptors and/or aryl hydrocarbon receptors (AhRs).[37,40] In contrast, the Phase II enzymes such as GST and NQO1 are induced through the action of Nrf2 transcription factor.[41,42,43] That means that the Phase II enzymes employ largely distinct mechanisms from those of Phase I enzymes.

In general, xenobiotics such as carcinogens are first metabolized by the action of phase I enzymes into reactive electrophilic metabolites and then conjugated by the action of phase II enzymes, which facilitates their excretion from the body. Therefore, the toxicological activities of chemical carcinogens are influenced by the balance between the phase I and phase II enzymes.[44] The present study has demonstrated clearly that ZER activates phase II enzymes, but not phase I enzymes, which changes the balance toward detoxification.

Judging from the magnitude of GST and QR augmentation, ZER exhibits approximately the same biological potential as BHA, the well-known phase II enzymes inducer.[12,13] As BHA has been proved to suppress various types of chemical carcinogenesis,[45] ZER may also possess anti-carcinogenic activity. Supporting the idea, augmentation of GST expression has been found to lower carcinogenetic risk in the prostate cells,[38] whereas its decline to increase the risk. Additionally, GST activation is considered to reduce the risk of colorectal, hepatic and esophageal cancers.[40] Thus, our observations herein of ZER enhancement of the GST activities are potent suggestion for its antitumor activity. In this connection, it has been shown recently that ZER is suppressive on NF-κB-related gene expression.[10] The genes affected include antiapoptotic and proliferative genes such as Bcl-2, Bcl-xL, cyclin D1 and c-Myc. The effect of ZER on NF-κB functions may further enhance the possible anticarcinogenic activity of ZER.

In summary, we have shown that treatment with ZER causes an elevation of the activities of phase II enzymes in mouse liver and small intestine, the first organs in con-

tact with ingested foods or chemicals. Importantly, we observed little damaging effect therein, consistent with the report that ZER is scarcely toxic.[10] These results suggested that ZER may be a promising chemoprotective agent.

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