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Modulation of Rat Hepatic and Pulmonary Cytochromes P450 and Phase II Enzyme Systems by Erucin, an Isothiocyanate Structurally Related to Sulforaphane

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Administration of dietary doses of the isothiocyanate erucin had no effect on rat hepatic cytochrome P450 activity or protein levels, but at higher doses a rise in CYP1A/B1 protein levels was evident. In lung, treatment with erucin, as well as sulforaphane, failed to modulate cytochrome P450 activities but elevated CYP1A/B1 protein levels. In liver, erucin stimulated quinone reductase activity accompanied by a rise in protein. Glutathione *S*-transferase activity was unaffected, but GSTα and GSTμ protein levels increased. In lung, both isothiocyanates increased quinone reductase paralleled by a rise in protein levels; at the higher dose both isothiocyanates elevated moderately GSTα levels. Hepatic microsomes converted both isothiocyanates to metabolites that impaired cytochrome P450 activity, which was antagonized by reduced glutathione. It may be concluded that erucin may protect against carcinogens by stimulating the detoxication of quinones but is unlikely to significantly influence reactive intermediate generation through modulation of cytochrome P450 activity.

KEYWORDS: Erucin; sulforaphane; isothiocyanates; chemoprevention; quinone reductase

INTRODUCTION

Isothiocyanates comprise a class of phytochemicals present in cruciferous vegetables and are considered to be promising human chemopreventive agents. Epidemiological studies have revealed the potential of these vegetables to protect against a number of cancers (*1*–*3*), and indeed individual isothiocyanates have been linked, through epidemiological studies, to lower cancer incidence at a number of sites (*4, 5*). Experimental evidence in animal models supports a chemopreventive role for these chemicals; isothiocyanates can antagonize the carcinogenicity of chemicals, including dietary carcinogens such as polycyclic aromatic hydrocarbons and heterocyclic amines (*6, 7*). Of importance are recent reports that major metabolites of isothiocyanates, such as mercapturates, retain the chemopreventive activity (*8*).

In vegetables isothiocyanates exist in the form of glucosinolates but are released following exposure to the enzyme myrosinase (β-thioglucoside glucohydrolase), which comes into contact with these compounds during the harvesting, chopping, and mastication of these vegetables. Moreover, glucosinolates that progress to the human intestine intact may be metabolized by microbial myrosinases, further contributing to the release of isothiocyanates (*9*). Isothiocyanates are well absorbed and achieve good bioavailability (*10*). Moreover, experimental evidence has been recently presented that the glucosinolates of isothiocyanates can also be absorbed, at least partly, intact (*11*).

The chemopreventive mechanism of action of isothiocyanates appears to be multifactorial, influencing all stages of chemical carcinogenesis, including impairment of the initiation stage (*3, 12*). Indeed, the consumption of cruciferous vegetables influenced the metabolism of carcinogenic heterocyclic amines in humans (*13*). One of the most extensively studied isothiocyanates is sulforaphane [1-isothiocyanato-4-(methylsulfinyl)butane] (**Figure 1**), encountered at substantial concentrations in broccoli, in which it exists as the glucosinolate glucoraphanin. Even when administered at dietary doses, sulforaphane stimulates detoxication enzymes such as quinone reductase (*14*), and at higher doses it also enhances the activity of other deactivating enzyme systems such as glutathione *S*-transferase (GST) (*15*). However, sulforaphane also acts at postinitiation stages, inducing apoptosis, cell cycle arrest, inhibition of histone deacetylase, and

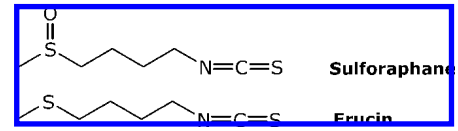

Figure 1. Chemical structures of erucin and sulforaphane.

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suppression of the conversion of lung benign tumors to carcinomas in mice (*8, 16*).

Erucin [1-isothiocyanato-4-(methylthio)butane] (**Figure 1**) is another isothiocyanate, structurally related to sulforaphane, being its sulfide analogue. It is found as the glucosinolate, glucoerucin, and is a major glucosinolate in rocket salad (roquette; *Eruca sativa*); moreover, there is experimental evidence to suggest that glucoraphanin can be reduced to glucoerucin by mammalian enzymes (*11*). Erucin itself is also an important metabolite of sulforaphane formed from the reduction of the sulfinyl group; similarly, sulforaphane is a principal metabolite of erucin, generated following sulfur oxidation (*11, 17*). Erucin appears to share many of the biological activities of sulforaphane related to its chemopreventive activity, in many cases being far more potent (*18, 19*).

Vegetables are usually cooked by boiling, and this procedure results in marked loss of glucosinolates, which leach into the cooking water, whereas other cooking processes such as steaming and microwaving have minimal effects (*20*). More importantly, cooking of vegetables denatures myrosinase, the enzyme that converts the glucosinolates to the active isothiocyanates (*21*). As a result of these two effects, the bioavailabilty of isothiocyanates is drastically decreased. Indeed, the bioavailability of isothiocyanates from broccoli markedly decreased after steaming (*22*). An advantage of rocket salad, the major source of the erucin glucosinolate, is that it is largely consumed raw and, consequently, glucosinolate levels and myrosinase activity are not adversely affected. As modulation of the xenobiotic-metabolizing enzyme systems, resulting in lower availability of the genotoxic metabolites, is a major mechanism of the anticarcinogenic effects of isothiocyanates, a study has been undertaken to evaluate the potential of erucin to modulate these enzyme systems in the liver of rats. Previous studies have been reported in murine hepatoma cells and in mice treated with erucin by gavage, focusing only on phase II enzyme systems (*23*) but, to our knowledge, this is the first study investigating the modulation of cytochrome P450 enzymes. Moreover, the ability of erucin to modulate carcinogen-metabolizing enzymes in the lung, a target tissue of isothiocyanates, has been investigated and compared to that of sulforaphane.

MATERIALS AND METHODS

Sulforaphane and erucin (LKT Laboratories, St. Paul, MN), NADPH, ethoxyresorufin, pentoxyresorufin, resorufin, 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene, glutathione reductase, peroxidase-linked anti-rabbit and anti-goat antibodies raised in rabbits (Sigma Co. Ltd., Poole, Dorset, U.K.), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Fluka, Buchs SG, Switzerland), 7-benzyloxyquinoline and 7-hydroxyquinoline (BD Biosciences, San Jose, CA), and 2-amino-3-methylimidazo-(4,5-*f*)quinoline (IQ) (Toronto Research Chemicals, Toronto, Canada) were all purchased. Rat anti-CYP1A1, recognizing both CYP1A1 and CYP1A2, anti-CYP1B1, anti-CYP2B, and anti-CYP3A2 antibodies were obtained from BD Biochemicals (Oxford, U.K.); antibodies to human GST P1-1, A1-1, and M1-1 (Calbiochem, Merck, U.K.) and antibody to human quinone reductase (abcam Cambridge, U.K.) were similarly purchased.

Male Wistar albino rats (about 180 g) were obtained from B&K Universal Ltd. (Hull, East Yorkshire, U.K.). The animals were housed at 22 ± 2 °C and 30−40% relative humidity in an alternating 12 h light/dark cycle with light onset at 7:00 a.m. Rats were randomly divided into four groups, each comprising five animals. One group served as control, two groups were maintained on drinking water containing 30 or 150 mg/L erucin, corresponding to approximate daily doses of 3 and 15 mg/kg (18.6 and 93.2 μmol/kg) respectively, and the final group was exposed to water containing sulforaphane (150 mg/L, approximate daily dose of 15 mg/kg, i.e., 88.2 μmol/kg); animals were maintained on these regimens for 10 days. At the end of this period, rats were killed by cervical dislocation, livers and lungs were immediately excised and homogenized, and postmitochondrial fractions were prepared by centrifugation (9000*g* for 20 min) and stored at −80 °C until use. When required, samples were thawed and resolved to microsomal and cytosolic fractions by centrifugation (105000*g* × 1 h). The dealkylations of methoxy- (*24*), ethoxy- (*25*), and pentoxyresorufin (*26*) and 7-benzyloxyquinoline (*27*) were determined using the microsomal fraction. The following assays were performed in the cytosol; quinone reductase using 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as substrate (*28*), glutathione *S*-transferase activity using as accepting substrates 1-chloro-2,4-dinitrobenzene, 1,2-dichloro-4-nitrobenzene (*29*), and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (*30*), and total glutathione (*31*). Protein concentration was determined in both fractions using bovine serum albumin as standard (*32*). Finally, to determine changes in enzyme protein expression, hepatic and pulmonary microsomal proteins from pooled animals were resolved by SDS-PAGE before being transferred electrophoretically to Hybond-P polyvinylidene difluoride membrane. Immunoblot analysis was carried out using antibodies to cytochrome P450 or phase II enzymes, followed by the appropriate peroxidase-labeled secondary antibody. Molecular markers were always run concurrently. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corp., Cambridge, U.K.).

To induce the hepatic CYP1A and CYP2B subfamilies of cytochrome P450, rats were treated with a single intraperitoneal dose of Aroclor-1254 (500 mg/kg), the animals being killed on the fifth day following administration. In studies aimed at establishing the effect of erucin (25 μM) on the in vitro O-dealkylation of ethoxyresorufin, microsomes from Aroclor-1254-induced animals were incubated with erucin or sulforaphane in the presence of NADPH for various periods of time, and reaction was initiated with the addition of the substrate. In studies aimed at studying the concentration-dependent effects, microsomes from Aroclor-1254-induced rats were incubated with a series of concentrations of erucin or sulforaphane in the presence of NADPH for 30 min, and reaction was initiated with the addition of the substrate.

Mutagenic activity induced by IQ was monitored using the Ames mutagenicity assay (*33*) in the presence of an activation system containing 10% (v/v) hepatic S9 preparations from control and erucin- and Aroclor-1254-treated rats and employing *Salmonella typhimurium* YG1024 as the indicator strain.

Statistical evaluation was carried out employing Student's *t* test.

RESULTS

Intake of neither erucin nor sulforaphane influenced animal body weight gain, and water consumption did not differ between control and isothiocyanate-treated animals (results not shown).

Treatment of rats with erucin failed to modulate the hepatic dealkylations of methoxy-, ethoxy-, or pentoxyresorufin, catalyzed selectively by CYP1A2, CYP1A1, and CYP2B, respectively, and of 7-benzoyloxyquinoline catalyzed by CYP3A (**Table 1**). Immunoblot analysis employing antibodies to rat CYP1A1 recognized a single band, presumably CYP1A2, the levels of which were modestly (30%) elevated by the higher dose of erucin (**Figure 2A**). A single band was also recognized by the antibodies to CYP1B1, and its expression increased (90%) following treatment of the animals with the higher dose of erucin (**Figure 2A**). Treatment with erucin failed to modulate the levels of the single band detected by anti-rat CYP2B1 and CYP3A2 antibodies (**Figure 2A**).

Cytosolic glutathione *S*-transferase activity in the liver, determined using 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, 1-chloro-2,4-dinitrobenzene, or 1,2-dichloro-4-nitrobenzene as substrates, and total glutathione levels were unaffected by the erucin treatment, whereas quinone reductase activity was stimulated at both dose levels (**Table 1**). Immunoblot analysis indicated that erucin, at both dose levels, up-regulated the levels of GSTα and GSTμ, as well as quinone reductase, but GSTπ was unaffected (**Figure 2B**).

Table 1. Effect of Treatment with Erucin on Rat Hepatic Cytochrome P450 and Phase II Enzyme Activities[a]

enzyme activity	control	erucin (low dose)	erucin (high dose)
methoxyresorufin *O*-demethylase (pmol/min/mg of protein)	23 ± 6	20 ± 2	16 ± 6
ethoxyresorufin *O*-deethylase (pmol/min/mg of protein)	30 ± 7	24 ± 3	20 ± 9
pentoxyresorufin *O*-depentylase (pmol/min/mg of protein)	3.3 ± 0.7	2.5 ± 0.5	3.1 ± 0.7
benzyloxyquinoline *O*-debenzylase (pmol/min/mg of protein)	327 ± 84	369 ± 60	291 ± 63
quinone reductase (nmol/min/mg of protein)	679 ± 298	1074 ± 125**	1270 ± 130***
glutathione *S*-transferase (1-chloro-2,4-dinitrobenzene) (nmol/min/mg of protein)	2137 ± 190	2181 ± 258	1947 ± 236
glutathione *S*-transferase (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) (nmol/min/mg of protein)	463 ± 112	414 ± 116	477 ± 116
glutathione *S*-transferase (1,2-dichloro-4-nitrobenzene) (nmol/min/mg of protein)	73 ± 5	67 ± 11	66 ± 11
total glutathione (mM)	5.13 ± 0.67	4.56 ± 0.51	4.76 ± 0.56

[a] Results are presented as mean ± SD for five rats. **, significantly different compared with control ($P < 0.01$). ***, significantly different compared with control ($P < 0.001$).

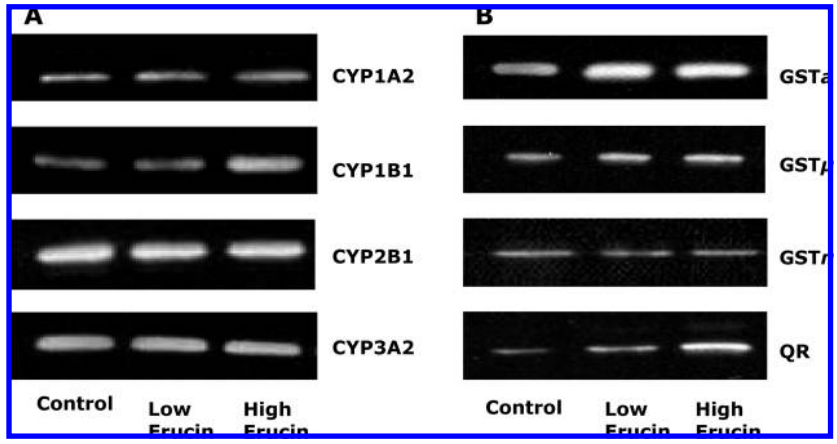

Figure 2. Modulation of rat hepatic cytochrome P450 and phase II enzymes by treatment with erucin.

Table 2. Effect of Treatment with Erucin and Sulforaphane on Rat Pulmonary Cytochrome P450 and Phase II Enzyme Activities[a]

enzyme activity	control	erucin (low dose)	erucin (high dose)	sulforaphane
ethoxyresorufin *O*-deethylase (pmol/min/mg of protein)	0.91 ± 0.35	0.71 ± 0.21	0.98 ± 0.27	0.71 ± 0.29
pentoxyresorufin *O*-depentylase (pmol/min/mg of protein)	3.26 ± 0.70	2.53 ± 0.50	3.09 ± 0.69	2.59 ± 1.02
quinone reductase (nmol/min/mg of protein)	1288 ± 298	1709 ± 238*	2526 ± 40**	2775 ± 497**
glutathione *S*-transferase (1-chloro-2,4-dinitrobenzene) (nmol/min/mg of protein)	24 ± 5	23 ± 4	32 ± 6	31 ± 6
glutathione *S*-transferase (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) (nmol/min/mg of protein)	2.84 ± 0.58	2.16 ± 0.68	3.45 ± 0.61	3.79 ± 0.78
total glutathione (mM)	0.82 ± 0.28	0.66 ± 0.08	0.82 ± 0.22	0.79 ± 0.09

[a] Rats were maintained on erucin- or sulforaphane-supplemented diets for 10 days. Results are presented as mean ± SD for five rats. *, significantly different compared with control ($P < 0.05$); **, significantly different compared with control ($P < 0.01$).

In lung, neither isothiocyanate modulated the O-dealkylation of the two alkoxyresorufins (**Table 2**); 7-benzyloxyquinoline demethylase was not detectable in this tissue. Immunoblot studies revealed that erucin, at both doses, as well as sulforaphane elevated pulmonary CYP1A1 protein levels (**Figure 3A**). Similarly, the higher erucin dose and sulforaphane increased the band recognized by antibody to CYP1B1 antibody by 80 and 70%, respectively (**Figure 3A**). No changes were evident in the case of CYP2B1 or CYP3A2. Glutathione *S*-transferase activity, whether monitored with 1-chloro-2,4-dinitrobenzene or with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, and total glutathione levels were not influenced by the isothiocyanate treatment. Quinone reductase activity, which was higher in the lung compared with the liver, was clearly elevated by the treatments with erucin or sulforaphane (**Table 2**). The increase in quinone reductase activity was paralleled by a similar rise in protein levels determined by immunoblot, being 165 and 150% higher in the animals treated with the high erucin dose and sulforaphane, respectively, in comparison with controls (**Figure 3B**). Erucin, at the higher dose, similar to sulforaphane, raised the levels of GSTα by 25−30%, whereas GSTπ and GSTμ were unaffected (**Figure 2B**).

Both isothiocyanates, following preincubation in the presence of NADPH, suppressed the hepatic O-deethylation of ethoxyresorufin in Aroclor-1254-induced hepatic microsomes to a similar degree (**Figure 4A**,**B**); the extent of inhibition was time-dependent with respect to preincubation time (**Figure 4C**). Reduced glutathione prevented the suppression of O-deethylation of ethoxyresorufin following preincubation with the isothiocyanate (**Figure 5**); oxidized glutathione had no effect (results not shown). Finally, in the absence of preincubation, neither compound, at least up to a concentration of 25 μM, influenced the O-deethylation of ethoxyresorufin (results not shown).

The bioactivation of the heterocyclic amine IQ to genotoxic intermediates in the Ames test was determined in the presence of hepatic postmitochondrial preparations from control and

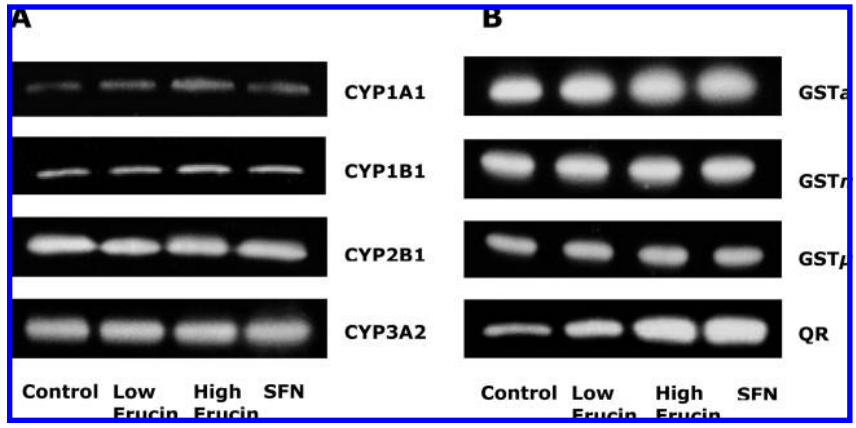

Figure 3. Modulation of rat pulmonary cytochrome P450 and phase II enzymes by treatment with erucin and sulforaphane.

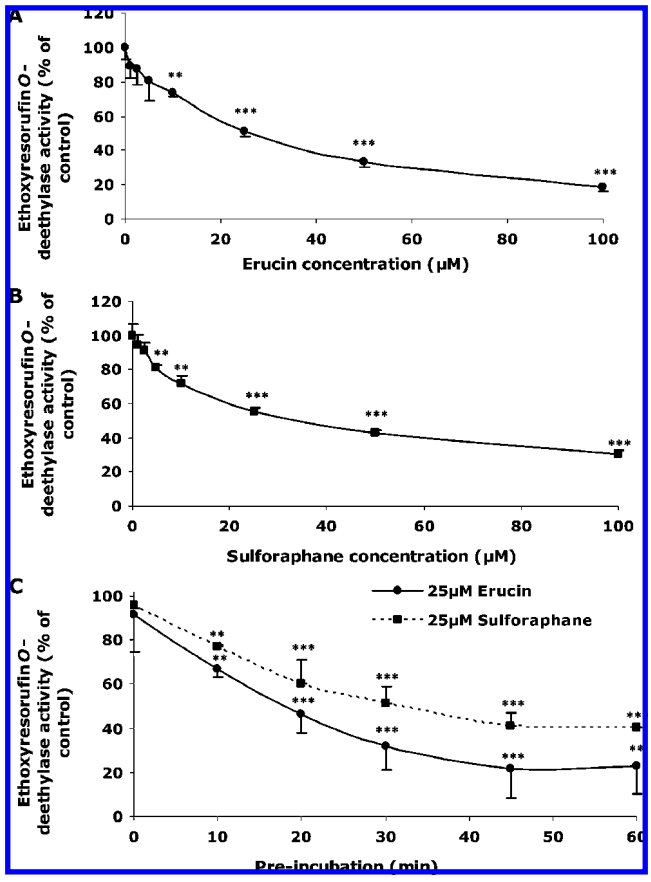

Figure 4. Effect of preincubation on the inhibition of the hepatic O-deethylation of ethoxyresorufin by erucin and sulforaphane. Results are presented as mean ± SD of triplicate determinations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

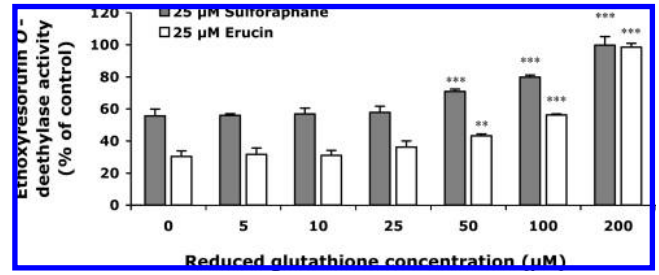

Figure 5. Effect of glutathione on the erucin- and sulforaphane-mediated inhibition of the O-deethylation of ethoxyresorufin. Results are presented as mean ± SD of triplicate determinations. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

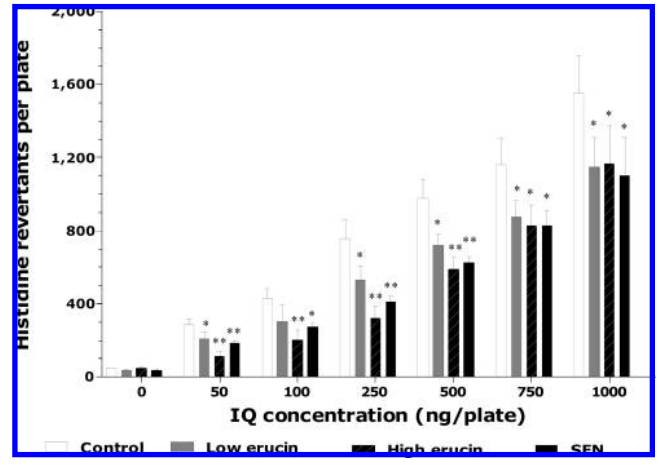

Figure 6. Effect of erucin treatment on the activation of IQ to mutagenic intermediates in the Ames test. Aroclor 1254-induced activation system (positive control) elicited a mutagenic response of 7562 ± 1240 revertants/plate at an IQ concentration of 1000 ng/plate. The spontaneous reversion rate of 48 ± 8 has already been subtracted. Results are presented as mean ± SD for triplicate plates. *, $P < 0.05$; **, $P < 0.01$.

erucin- and sulforaphane-pretreated rats. A concentration-dependent mutagenic response was evident in the presence of all activation systems; treatment with either isothiocyanate caused a modest decrease in mutagenic response (**Figure 6**).

DISCUSSION

One of the major mechanisms through which isothiocyanates are believed to elicit their anticarcinogenic effects is by limiting the amount of genotoxic metabolites of chemical carcinogens by either stimulating their detoxication and/or impairing their generation (*3*). Consequently, the aim of this study was to evaluate the potential of the isothiocyanate erucin to modulate glutathione *S*-transferase and quinone reductase, two pivotal enzymes in the detoxication of reactive intermediates and prevention of oxidative stress, and cytochromes P450, the most important enzyme in the generation of these intermediates; studies were conducted in the liver, the principal site of metabolism, and lung, a target tissue for the anticarcinogenic effect of isothiocyanates (*9*). The effects of erucin on pulmonary enzymes were compared with those of sulforaphane, as the influence of the latter has not been studied in this tissue following exposure to dietary doses. It has been established that sulforaphane is metabolized to erucin in the rat, and erucin can be readily formed from the reduction of sulforaphane (*11, 17*).

Previous studies in mice have indicated the potential of erucin and sulforaphane, at least at high doses administered by gavage, to stimulate quinone reductase and glutathione *S*-transferase activities in the liver and other tissues (*23*), but to our knowledge this is the first study evaluating the potential of this isothiocyanate to modulate cytochrome P450 enzymes. Both isothiocyanates were given to rats in the drinking water, and a dose was employed that corresponds to human intake. Because, to our knowledge, the human consumption levels of erucin or glucoerucin have not been defined, the doses employed in these studies was based on the consumption of sulforaphane, a structurally similar isothiocyanate. A 250 g serving of fresh broccoli will release 93−187 mg of sulforaphane (*34*), so that the intake for a 70 kg individual would be 1.3−2.7 mg/kg. The lower dose of erucin employed in the current study of approximately 3 mg/kg represents such dietary level of intake.

None of the cytochrome P450 activities studied were modulated by treatment with erucin in the liver or lung or with sulforaphane in the lung. In the lung, however, both compounds elevated the levels of the band recognized by the antibody to CYP1A1. CYP1B1 could not be monitored at the activity level as a selective substrate is not available, but immunoblot analysis indicated that treatment with erucin, at the higher dose, elevated the levels of this enzyme in the liver. In the lung, both isothiocyanates led to an increase in the single band detected by the CYP1B1 antibody. Neither compound had any effect on CYP2B or CYP3A2 protein levels. Up-regulation of the CYP1 family, albeit at the mRNA level, has been reported following exposure of HepG2 cells to 4-methylthio-3-butenyl isothiocyanate (*35*).

Erucin, at both dose levels, gave rise to a significant increase in hepatic quinone reductase activity as previously described for sulforaphane (*14*). For both tissues, Western blot analysis indicated that this was due to increased cytosolic concentration of quinone reductase. Contrary to the present observations, exposure of rats to phenethyl isothiocyanate failed to enhance this activity in the lung (*36*), highlighting the importance of the side chain; it is of interest that sulforaphane and erucin have an aliphatic side chain, whereas in the case of phenethyl isothiocyanate it is aromatic. Glutathione *S*-transferase activity was monitored using three substrates; 1-chloro-2,4-dinitrobenzene is a substrate for a number of the cytosolic transferases (*37*). The much higher activity in the liver compared with the lung reflects the higher capacity of the former tissue to detoxicate electrophiles. Both compounds failed to influence activity in lung and, similarly, in the liver erucin had no effect. 1,2-Dichloro-4-nitrobenzene is a substrate associated with the μ-class, but no activity was detectable in lung as a result of poor expression in this tissue (*37*). Hepatic activity was not influenced by erucin treatment, although a rise in protein levels was clearly visible. Finally, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole has been reported to be selective for the α-class (*30*), and the very low activity in the lung demonstrates the poor expression of this class in this tissue (*37*). A rise in GST activity was not seen with any of the three accepting substrates studied, whereas GSTα and GSTμ were up-regulated at the protein level. Similarly, in lung no change in activity was manifested by treatment with either isothiocyanate, but GSTα levels increased; GSTπ was not modulated by either isothiocyanate in either tissue. Thus, the effects of isothiocyanates on the GST system are both compound and tissue specific.

As isothiocyanates, with either aliphatic or aromatic side chain, can be metabolically converted to intermediates that cause cytochrome P450 inhibition, the ability of erucin to act as a mechanism-based inhibitor was evaluated and compared to that of sulforaphane. The two isothiocyanates did not differ in their ability to suppress, in a time-dependent manner, the O-deethylation of ethoxyresorufin. The fact that inhibition was prevented by reduced, but not oxidized, glutathione concords with the generation of an electrophilic metabolite(s). A more reactive isocyanate, derived from the cytochrome P450-mediated isothiocyanate oxidation (*38*), has been postulated as the possible entity that interacts and inactivates cytochromes P450. Isocyanates are known to be capable of modifying proteins. Indeed, α-naphthylisothiocyanate was converted by rat liver microsomes, in the presence of NADPH, to metabolites that bound irreversibly to microsomal proteins (*39*).

To assess whether the erucin treatment had any impact on the ability of liver microsomes to bioactivate chemical carcinogens, the metabolic conversion of the heterocyclic amine 2-amino-3-methylimidazo-(4,5-*f*)quinoline (IQ) to mutagens was investigated using the Ames mutagenicity assay. This compound was used as a model carcinogen as previous studies showed that sulforaphane inhibits the genotoxicity and DNA adduct formation of heterocyclic amines in cell cultures in vitro (*40, 41*). Erucin treatment caused a modest decrease in the bioactivation of IQ, but no clear concentration-dependent effect was evident. Sulforaphane had a similar, modest effect as we previously reported (*14*).

In summary, the present studies have demonstrated that following intake of erucin, at a dose corresponding to human intake, the only enzyme modulated of those studied was quinone reductase; an increase was seen in both liver and lung. No cytochrome P450 enzyme was perturbed by the erucin treatment, implying that attenuated generation of genotoxic intermediates is unlikely to be a chemopreventive mechanism at dietary dose levels. These observations allow us to conclude that erucin may exert a chemopreventive effect by affording protection against the carcinogenicity of quinones, such as those formed by the oxidation of polycyclic aromatic hydrocarbons. At higher doses, which may be achieved by the consumption of dietary supplements, the expression of other enzymes was modulated, at least at the protein level. In general, no marked differences were observed between erucin and sulforaphane. However, as the major source of erucin is rocket salad, which is largely consumed uncooked, it may prove to be a more promising chemopreventive agent than sulforaphane as it is likely to achieve a higher bioavailability. The principal sources of sulforaphane are vegetables that are consumed cooked, resulting in lower conversion of the glucosinolate to the isothiocyanate. Finally, to our knowledge, this is the first paper to report the ability of dietary isothiocyanates to up-regulate CYP1B1 expression in the liver and lung.

LITERATURE CITED

(1) van Poppel, G.; Verhoeven, D. T.; Verhagen, H.; Goldbohm, R. A. *Brassica* vegetables and cancer prevention. Epidemiology and mechanisms. *Adv. Exp. Med. Biol.* **1999**, *472*, 159–168.

(2) Ambrosone, C. B.; McCann, S. E.; Freudenheim, J. L.; Marshall, J. R.; Zhang, Y.; Shields, P. G. Breast cancer risk in premenopausal women is inversely associated with consumption of broccoli, a source of isothiocyanates, but is not modified by GST genotype. *J. Nutr.* **2004**, *134*, 1134–1138.

(3) Zhang, Y. Cancer-preventive isothiocyanates: measurement of human exposure and mechanism of action. *Mutat. Res.* **2004**, *555*, 173–190.

(4) Spitz, M. R.; Duphorne, C. M.; Detry, M. A.; Pillow, P. C.; Amos, C. I.; Lei, L.; de Andrade, M.; Gu, X. J.; Hong, W. K.; Wu, X. F. Dietary intake of isothiocyanates: Evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.* **2000**, *9*, 1017–1020.

(5) Lin, H. J.; Probst-Hensch, N. M.; Louie, A.; Kau, I. H.; Witte, J. S.; Ingles, S. A.; Frankl, H. D.; Lee, E. R.; Haile, R. W. Glutathione transferase null genotype, broccoli, and lower preva-

lence of colorectal adenomas. *Cancer Epidemiol. Biomarkers Prev.* **1998**, *7*, 647–652.
(6) Hecht, S. S. Inhibition of carcinogenesis by isothiocyanates. *Drug Met. Rev.* **2000**, *32*, 395411.
(7) Kuroiwa, Y.; Nishikawa, A.; Kitamura, Y.; Kanki, K.; Ishii, Y.; Umemura, T.; Hirose, M. Protective effects of benzyl isothiocyanate and sulforaphane but not resveratrol against initiation of pancreatic cancers in hamsters. *Cancer Lett.* **2006**, *241*, 275–280.
(8) Conaway, C. C.; Wang, C.-X.; Pittman, B.; Yang, Y.-M.; Schwartz, J. E.; Tian, D.; McIntee, E. J.; Hecht, S. S.; Chung, F.-L. Phenethyl isothiocyanate and sulforaphane and their *N*-acetylcysteine conjugates inhibit malignant progression of lung adenomas induced by tobacco carcinogens in A/J mice. *Cancer Res.* **2005**, *65*, 8548–8557.
(9) Getahun, S. M.; Chung, F.-L. Conversion of isothiocyanates in humans after ingestion of cooked watercress. *Cancer Epidemiol. Biomarkers Prev.* **1999**, *8*, 447–451.
(10) Hanlon, N.; Coldham, N.; Gielbert, A.; Kuhnert, N.; Sauer, M. J.; King, L. J.; Ioannides, C. Absolute bioavailability and dose-dependent pharmacokinetic behaviour of dietary doses of the chemopreventive isothiocyanate sulforaphane in rat. *Br. J. Nutr.* **2008**, *99*, 559–564.
(11) Bheemreddy, R. M.; Jeffery, E. H. The metabolic fate of purified glucoraphanin in F344 rats. *J. Agric. Food Chem.* **2007**, *55*, 2861–2866.
(12) Thornalley, P. J. Isothiocyanates: mechanism of cancer chemopreventive action. *Anti-Cancer Drugs* **2002**, *13*, 331–338.
(13) Walters, D. G.; Young, P. J.; Agus, C.; Knize, M. G.; Boobis, A. R.; Gooderham, N. J.; Lake, B. G. Cruciferous vegetable consumption alters the metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) in humans. *Carcinogenesis* **2004**, *25*, 1659–1669.
(14) Yoxall, V.; Kentish, P.; Coldham, N.; Kuhnert, N.; Sauer, M. J.; Ioannides, C. Modulation of hepatic cytochromes P450 and phase II enzymes by dietary doses of sulforaphane in rats: Implications for its chemopreventive activity. *Int. J. Cancer* **2005**, *117*, 356–362.
(15) Zhang, Y.; Talalay, P. Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. *Cancer Res.* **1998**, *58*, 4632–4639.
(16) Fimognari, C.; Hrelia, P. Sulforaphane as a promising molecule for fighting cancer. *Mutat. Res.* **2007**, *635*, 90–104.
(17) Kassahun, K.; Davis, M.; Hu, P.; Martin, B.; Baillie, T. Biotransformation of the naturally occurring isothiocyanate sulforaphane in the rat: identification of phase I metabolites and glutathione conjugates. *Chem. Res. Toxicol.* **1997**, *10*, 1228–1233.
(18) Jakubíková, J.; Sedlák, J.; Mithen, R.; Bao, Y. Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzyme and MRP2 transcription, G_2/M arrest and cell death in Caco-2 cells. *Biochem. Pharmacol.* **2005**, *69*, 1543–1552.
(19) Munday, R.; Munday, C. M. Induction of phase II detoxification enzymes in rats by plant-derived isothiocyanates: comparison of allyl isothiocyanate with sulforaphane and related compounds. *J. Agric. Food Chem.* **2004**, *52*, 1867–1871.
(20) Song, L.; Thornalley, P. J. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food Chem. Toxicol.* **2007**, *45*, 216–224.
(21) Rungapamestry, V.; Duncan, A. J.; Fuller, Z.; Ratcliffe, B. Effect of meal composition and cooking duration on the fate of sulforaphane following consumption of broccoli by healthy human subjects. *Br. J. Nutr.* **2007**, *97*, 644–652.
(22) Conaway, C. C.; Getahun, S. M.; Liebes, L. L.; Pusateri, D. J.; Topham, D. K. W.; Botero-Omary, M.; Chung, F.-L. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr. Cancer* **2001**, *38*, 468–478.
(23) Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. A major inducer of anticarcinogenic protective enzymes in broccoli: isolation and elucidation of structure. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403.
(24) Burke, M. D.; Mayer, R. T. Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal and cytochrome P450 binding of phenoxazone and a homologous series of its n-alkyl ethers (alkoxyresorufins). *Chem.−Biol. Interact.* **1983**, *45*, 243–258.
(25) Burke, M. D.; Mayer, R. T. Ethoxyresorufin; direct fluorimetric assay of a microsomal O-dealkylation which is preferentially inducible by 3-methylcholanthrene. *Drug Metab. Dispos.* **1974**, *2*, 583–588.
(26) Lubet, R. A.; Mayer, R. T.; Cameron, J. W.; Nims, R. N.; Burke, M. D.; Wolf, T.; Guengerich, F. Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome(s) P450 by phenobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys.* **1985**, *238*, 43–48.
(27) Stresser, D. M.; Turner, S. D.; Blanchard, A. P.; Miller, V. P.; Crespi, C. L. Cytochrome P450 fluorimetric substrates: identification of isoform-selective probes for rat CYP2D2 and human CYP3A4. *Drug Metab. Dispos.* **2002**, *30*, 845–852.
(28) Prohaska, H. J.; Santamaria, A. B. Direct measurement of NAD(P)H:quinone reductase from cells cultured in microtiter wells: a screening assay for anticarcinogenic enzyme inducers. *Anal. Biochem.* **1988**, *169*, 328–336.
(29) Habig, W. H.; Pabst, M. J.; Jakoby, W. B. Glutathione *S*-transferase, the first enzymic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139.
(30) Ricci, G.; Caccuri, A.; Bello, M.; Pastore, A.; Piemonte, F.; Federici, G. Colorimetric and fluorometric assays of glutathione-*S*-transferase based on 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole. *Anal. Biochem.* **1994**, *218*, 463–465.
(31) Akerboom, T. P. H.; Sies, H. Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. *Methods Enzymol.* **1981**, *7*, 373–382.
(32) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* **1976**, *2*, 248–254.
(33) Maron, M. D.; Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.
(34) Howard, L. A.; Jeffery, E. H.; Wallig, M. A.; Klein, B. P. Retention of phytochemicals in fresh and processed broccoli. *J. Food Sci.* **1997**, *62*, 1098–1104.
(35) Hanlon, P. R.; Webber, D. M.; Barnes, D. M. Aqueous extract of the Spanish black raddish (*Raphanus sativus* L. Var. *niger*) induces detoxification enzymes in the HepG2 hepatoma cell line. *J. Agric. Food Chem.* **2007**, *55*, 6439–6446.
(36) Guo, Z.; Smith, T. J.; Wang, E.; Sadrieh, N.; Ma, Q.; Thomas, P. E.; Yang, C. S. Effect of phenethyl isothiocyanate, a carcinogenesis inhibitor, on xenobiotic-metabolizing enzymes and nitrosamine metabolism in rats. *Carcinogenesis* **1992**, *13*, 2205–2210.
(37) Sherrat, P. J.; Hayes, J. D. Glutathione S-transferases. In *Enzyme Systems That Metabolise Drugs and Other Xenobiotics*; Ioannides, C., Ed.; Wiley: Chichester, U.K., 2002; pp 319−352.
(38) Lee, M. S. Oxidative conversion of isothiocyanates to isocyanates by rat liver. *Environ. Health Perspect.* **1994**, *102*, 115–118.
(39) El-Hawari, A. M.; Plaa, G. L. α-Naphthylisothiocyanate (ANIT) hepatotoxicity and irreversible binding to rat liver microsomes. *Biochem. Pharmacol.* **1977**, *26*, 1857–1866.
(40) Barcelo, S.; Mace, K.; Pfeifer, A. M. A.; Chipman, J. K. Production of DNA strand breaks by *N*-nitrosodimethylamine and 2-amino-3-methylimidazo(4,5-*f*)quinoline in THLE cells expressing human CYP isoenzymes and inhibition by sulforaphane. *Mutat. Res.* **1998**, *402*, 111–120.
(41) Bacon, J. R.; Williamson, G.; Garner, R. C.; Lappin, G.; Langouët, C.; Bao, Y. Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. *Carcinogenesis* **2003**, *24*, 1903–1911.

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