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Characterization of the Temporal Induction of Hepatic Xenobiotic-Metabolizing Enzymes by Glucosinolates and Isothiocyanates: Requirement for at Least a 6 h Exposure To Elicit Complete Induction Profile

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ABSTRACT: A mechanism of action of chemopreventive glucosinolates/isothiocyanates, established largely in vitro, is to modulate carcinogen-metabolizing enzymes. Extrapolation in vivo involves relating in vitro concentrations to plasma/tissue concentrations attained in vivo, thus assuming that even transient exposure modulates enzyme activity. To test this hypothesis, precision-cut rat liver slices were incubated with glucosinolates for up to 24 h, and the O-dealkylation of methoxyresorufin and ethoxyresorufin was determined; increased activities were observed only at incubations of at least 6 h. To evaluate phase II enzymes, isothiocyanates, namely, sulforaphane, erucin, and phenethyl isothiocyanate, were similarly incubated; quinone reductase increased after incubation for 6 h or longer. When glutathione *S*-transferase was monitored, the phenethyl isothiocyanate-manifested rise necessitated at least a 6 h incubation, whereas in the case of sulforaphane and erucin, the activity was elevated after only 2 h. It is inferred that a rise in carcinogen-metabolizing enzymes by glucosinolates/isothiocyanates necessitates tissue exposure of at least 6 h.

KEYWORDS: *glucoraphanin, glucosinolates, isothiocyanates, phenethyl isothiocyanate, sulforaphane*

INTRODUCTION

Strong epidemiological evidence has linked the consumption of cruciferous vegetables, such as broccoli, to lower cancer incidence at a number of sites,[1−4] and this effect is believed to be due to glucosinolates that are present in these vegetables in substantial amounts.[5] When these vegetables are disturbed, for example, during mastication, the enzyme myrosinase (*β*-thioglucoside glucohydrolase) is released and converts glucosinolates to the corresponding isothiocyanates, which are believed to mediate most of their biological activity; microbial myrosinase in the human intestine also contributes to this breakdown.[6]

Experimental evidence has been published indicating that a number of mechanisms are likely to underpin the chemopreventive activity of isothiocyanates, including modulation of carcinogen-metabolizing enzyme systems so as to facilitate their detoxification at the expense of activation, thus attenuating the availability of genotoxic metabolites.[7−9] Moreover, isothiocyanates also act at the postinitiation stages of carcinogenesis, inducing apoptosis, cell cycle arrest, and inhibition of histone deacetylase and suppressing the conversion of benign tumors to carcinomas.[10,11]

The vast majority of experimental studies carried out to define the chemopreventive action of glucosinolates/isothiocyanates have been performed in animal cells/tissues either in vitro or ex vivo. To extrapolate such findings to the in vivo situation and to further relate these to humans, the accepted process is to relate the concentrations used in vitro to the relevant plasma/tissue concentrations that are achieved in vivo. The approach is to relate the in vitro biologically active concentrations to maximum plasma concentrations (C_{max}) that are attained following therapeutic or dietary intake. Inherent to such an approach is the assumption that even transient tissue exposure to the biologically active concentrations is sufficient for a complete in vivo response to be manifested. To test this assumption in relation to glucosinolates/isothiocyanates, precision-cut rat liver slices were incubated with these compounds for different periods of time, and their ability to modulate carcinogen-metabolizing enzyme systems was investigated. The objective was to delineate the minimum period of exposure that is necessary for a biological response to be elicited.

MATERIALS AND METHODS

Chemicals. Phenethyl isothiocyanate, ethoxyresorufin, methoxyresorufin, resorufin, 1-chloro-2,4-dinitrobenzene (CDNB), antimouse and antigoat antibodies (Sigma Co. Ltd., Poole, Dorset, United Kingdom), *R*,*S*-sulforaphane and erucin (LKT Laboratories, MN), anti-CYP1A1 (AMS Biotechnology, Abingdon, United Kingdom), and anti-CYP1A2 (Chemicon International Inc., Hampshire, United Kingdom) were all purchased. Antibodies to human quinone reductase, lactate dehydrogenase, and *β*-actin were obtained from

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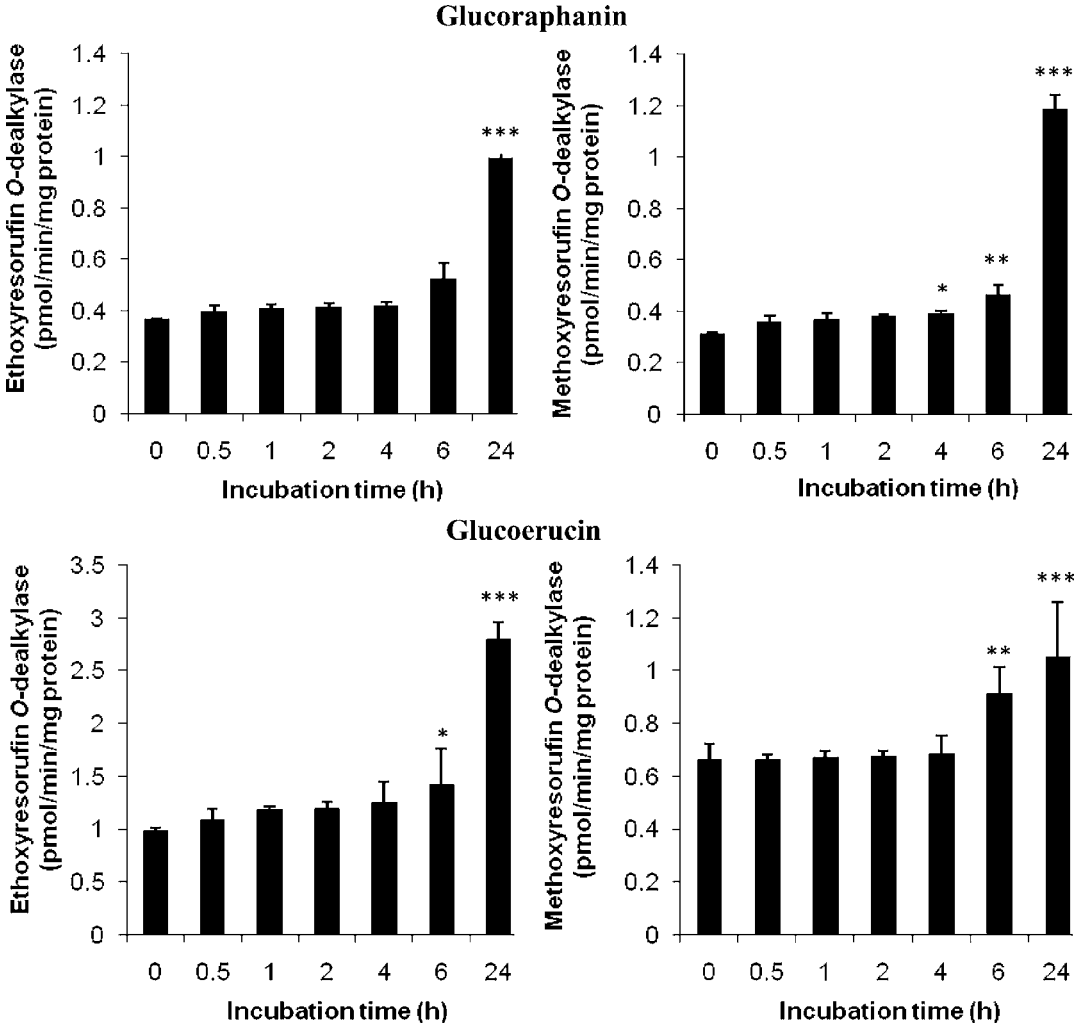

Figure 1. Effect of incubation time on the up-regulation of methoxy- and ethoxyresorufin *O*-deethylase activities by glucosinolates. Precision-cut rat liver slices were incubated with either glucoraphanin or glucoerucin (2.5 μM) for 0.5−24 h. At the end of the incubation, slices were pooled, microsomes were isolated, and the O-dealkylation of methoxy- and ethoxyresorufin was determined. Results are presented as means ± SDs for three pools of slices, each comprising 10 slices. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

abcam (Cambridge, United Kingdom), antibody to GSTα was from Calbiochem (Lutterworth, United Kingdom), and donkey antigoat and goat antirabbit antibodies were from Santa Cruz Biotechnology (CA). Glucoraphanin and glucoerucin were isolated from *Brassica oleracea* L. var. *acephala sabellica* (Cavolo nero di Toscana) and *Eruca sativa* ripe seeds, respectively, supplied by SUBA & UNICO (Longiano, Italy), according to a procedure developed at CRA-CIN of Bologna, Italy, as previously described.[12] The seeds were ground to a fine powder and defatted using hexane. Following removal of the solvent, each glucosinolate was extracted using boiled 70% ethanol to deactivate rapidly the endogenous myrosinase and homogenized using an Ultraturrax homogenizer at a medium speed for 15 min. The resulting homogenate was centrifuged at 17700*g* for 30 min, and the isolation of glucosinolate from the extract was carried out by one-step anion-exchange chromatography, employing DEAE-Sephadex A-25 (Amersham Biosciences, Milan, Italy). The glcosinolate was eluted using potassium sulfate (0.2 M), and the solution was concentrated to dryness using a rotary evaporator at 60−70 °C under vacuum. The glucosinolate was then solubilized by three subsequent extractions with about 200 mL in total of boiling methanol, and the alcoholic extract was filtered and concentrated to 15−20% of the initial volume. The solution was warmed and slowly added, dropwise, to 200 mL of 99.9% ethanol that was previously cooled to −20 °C. This led to the precipitation of a white powder. After centrifugation, the solid glucosinolate (as potassium salt) was dried, and further purification was carried out by gel filtration using an XK 26/100 column packed with Sephadex G10 (Amersham Biosciences, Milan, Italy) connected to a AKTA-FPLC (Amersham Biosciences, Milan, Italy), using high purity water as eluent. Fractions containing pure GL were pooled, freeze-dried, and sealed under vacuum to prevent moisture uptake by the highly hygroscopic compound. Both glucosinolates were characterized by ^{1}H and ^{13}C NMR spectroscopy, and purity (95−99%) was assayed by high-performance liquid chromatography (HPLC) analysis of the desulfo-derivative according to the ISO 9167-1 method (EEC Regulation 1864/90, 1990).

Preparation and Incubation of Precision-Cut Rat Liver Slices. Male Wistar albino rats (200−250 g) were obtained from Charles River UK Ltd. (Margate, Kent, United Kingdom). The animals were housed at 22 ± 2 °C, 30−40% relative humidity, in an alternating 12 h light:dark cycle with light onset at 07.00 h. Rat liver slices (200−300 μm) were prepared from 8 mm cylindrical cores using a Krumdieck tissue slicer (Alabama Research and Development Corporation, Munsford, AL) as previously described.[13] The multiwell plate procedure, using 12-well culture plates, was used to culture the slices in the presence of phenethyl isothiocyanate (1 μM), erucin (10 μM), sulforaphane (10 μM), glucoraphanin (2.5 μM), or glucoerucin (2.5 μM). One slice was placed in each well, in 1.5 mL of culture medium. Slices were incubated under sterile conditions for up to 24 h on a reciprocating plate shaker housed in a humidified incubator, at a temperature of 37 °C, and under an atmosphere of 95% air/5% CO_2.

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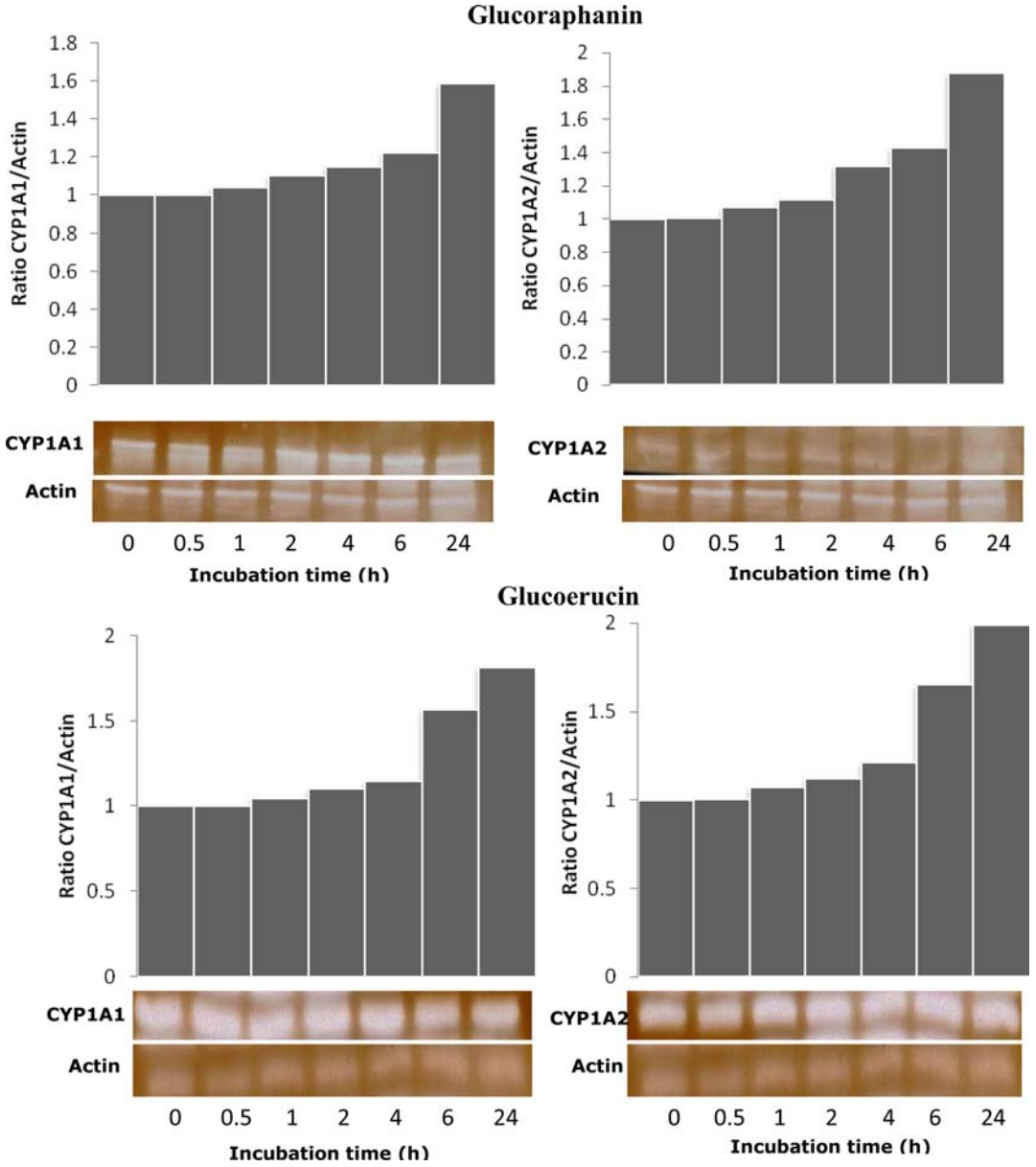

Figure 2. Effect of incubation time on up-regulation of CYP1A1 and CYP1A2 expression by glucosinolates. Precision-cut rat liver slices were incubated with either glucoraphanin or glucoerucin (2.5 μM) for 0.5−24 h. At the end of the incubation, slices were pooled, and microsomes were isolated. The immunoblot analysis was carried out by exposure to mouse antirat CYP1A1 and CYP1A2 followed by the appropriate peroxidase-labeled secondary antibody. Each lane was loaded with 30 μg of total protein. The blots were stripped and reprobed with anti-β-actin antibody to normalize for differences in protein loading.

The slices were initially preincubated for 30 min to slough off any dead cells due to slicing. Subsequently, slices were incubated for 0, 0.5, 1, 2, 4, and 6 h in the presence of the appropriate glucosinolate/isothiocyanate; at the end of the incubation period, slices were removed and placed into fresh medium lacking the glucosinolate/isothiocyanate and incubated for a further period so that the total incubation time was always 24 h. Finally, a set of slices were incubated with the glucosinolate/isothiocyanate for the complete 24 h period. Three different slice pools, each comprising 10 rat liver slices, were used in each of the seven time points to obtain sufficient material for all of the analyses to be performed, so that the total number of slices used per compound was 210.

Enzyme Assays. Following incubation, slices were removed from culture media, homogenized in 1.0 mL of 0.1 M Tris-HCl buffer, pH 7.4, and postmitochondrial supernatants were prepared and stored at −80 °C. When required, microsomes and cytosol were isolated by centrifugation (105000g × 1 h). The dealkylations of methoxy-[14] and ethoxyresorufin[15] were determined in the microsomal fraction. The incubation mixture comprised 0.1 M Tris-HCl buffer, pH 7.8 (1.85 mL), 0.1 mM methoxyresorufin/ethoxyresorufin (0.01 mL), and microsomes (0.10 mL); the reaction was initiated by the addition of 50 mM NADPH (0.01 mL), and the generation of resorufin at 37 °C was monitored spectrofluorimetrically for 10 min. The following determinations were carried out in the cytosolic fraction: quinone reductase (NQO1)[16] and glutathione S-transferase activity[17] monitored using CDNB. For the determination of quinone reductase activity, the incubation mixture comprised 25 mM Tris-HCl conatining 0.083% Tween 20 (0.80 mL), 100 mM 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide in 70% ethanil (0.006 mL), 50 mM NADPH (0.006 mL), and cytosolic fraction (0.050 mL); the reaction was inititated by the addition of 10 mM menadione (0.006 mL), and the production of blue formazan followed for a minute in a dual beam spectrophotometer. For the determination of glutathione S-transferase activity, the incubation mixture comprised

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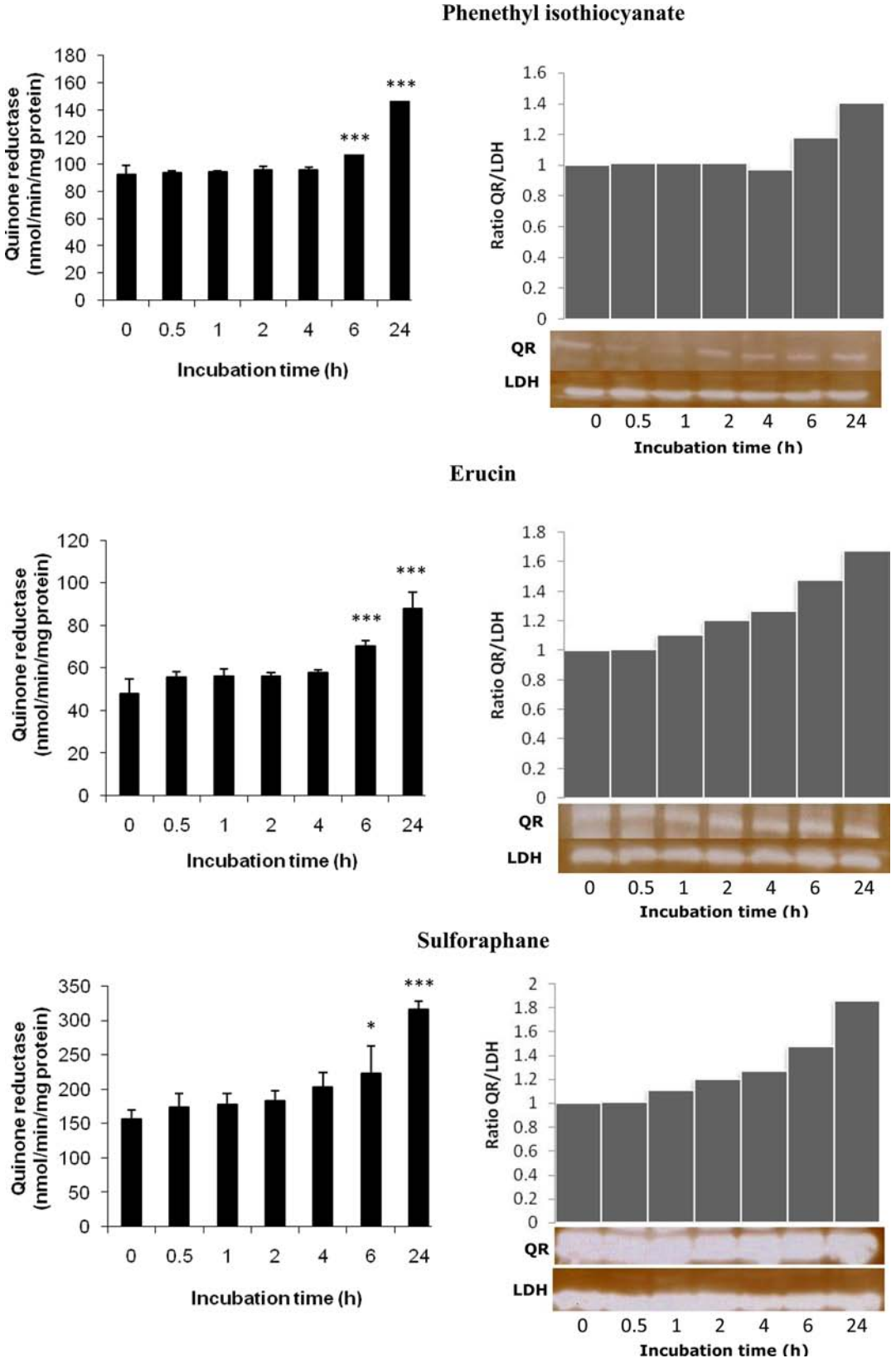

Figure 3. Influence of incubation time on the isothiocyanate-mediated up-regulation of quinone reductase activity and expression. Precision-cut rat liver slices were incubated with either sulforaphane (10 μM), erucin (10 μM), or phenethyl isothiocyanate (1 μM) for 0.5−24 h. At the end of the incubation, slices were pooled, the cytosolic fraction was isolated, and the quinone reductase activity was determined. The immunoblot analysis was carried out by exposure to rabbit antirat NQO1 primary antibodies followed by the appropriate peroxidase-labeled secondary antibody. Each lane was loaded with 30 μg of total protein. The blots were stripped and reprobed with anti-LDH antibody to normalize for differences in protein loading. Activity results are presented as means ± SDs for three pools of slices, each comprising 10 slices. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

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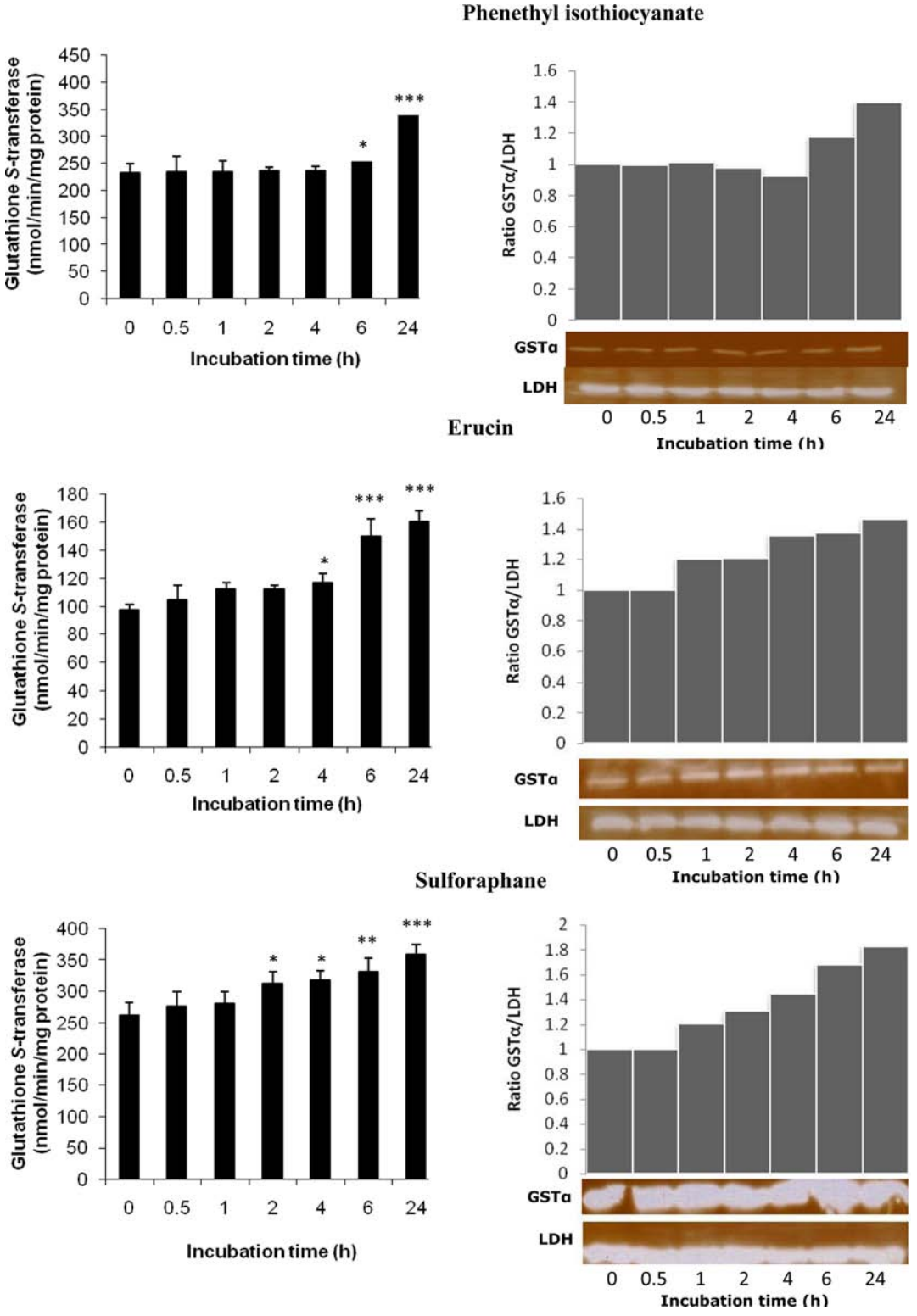

Figure 4. Influence of incubation time on the isothiocyanate-mediated up-regulation of glutathione *S*-transferase activity and expression. Precision-cut rat liver slices were incubated with either sulforaphane (10 μM), erucin (10 μM), or phenethyl isothiocyanate (1 μM) for 0.5−24 h. At the end of the incubation, slices were pooled, the cytosolic fraction was isolated, and the glutathione *S*-transferase activity was determined using CDNB as the substrate. The immunoblot analysis was carried out by exposure to rabbit antirat GSTA1-1 primary antibodies followed by the appropriate peroxidase-labeled secondary antibody. Each lane was loaded with 30 μg of total protein. The blots were stripped and reprobed with anti-LDH antibody to normalize for differences in protein loading. Activity results are presented as means ± SDs for three pools of slices, each comprising 10 slices. *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

0.1 M potassium phosphate buffer, pH 7.5 (1.0 mL), 25 mM CDNB in 70% ethanol (0.05 mL), 25 mM reduced glutathione (0.25 mL), and cytosol (0.05 mL); the formation of the glutathione conjugate was monitored for 1 min in a dual beam spectrophotometer. The protein

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concentration was determined in both cellular subfractions using bovine serum albumin as the standard.[18] Finally, to monitor whether an increase in enzyme activities reflects, at least partly, a rise in enzyme availability, Western blot analysis was performed by pooling all slices (a total of 30 slices). Hepatic microsomal or cytosolic proteins from pooled slices were loaded on to 10% (w/v) SDS-PAGE and then transferred electrophoretically to Hybond-P polyvinylidene difluoride membrane. The immunoblot analysis of rat proteins was carried out by exposure to the primary antibodies followed by the appropriate peroxidase-labeled secondary antibody. Lactate dehydrogenase (LDH) and β-actin antibodies were used as the housekeeping proteins for the cytosolic and microsomal proteins, respectively, to normalize protein loading. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corporation, Cambridge, United Kingdom), with the control band designated as 100%.

Statistical Evaluation. Enzyme activities are presented as means ± standard deviations (SDs) of three pools, each comprising 10 slices. Statistical evaluation was carried out by one-way analysis of variance (ANOVA) followed by the Dunnett's test.

RESULTS

Studies with Glucosinolates. The glucosinolates glucoerucin and glucoraphanin, when incubated with precision-cut rat liver slices, elevated the O-dealkylation of methoxy- and ethoxyresorufin, markers for CYP1-dependent oxidative metabolism; the effect was manifested following exposure of liver slices to these compounds for 6 or 24 h (Figure 1). The rise in activity was more pronounced after the longer incubation. Both compounds increased CYP1A1 and CYP1A2 apoprotein levels, but a clear rise in expression was seen only after a 6 h incubation in the case of glucoerucin and a 24 h incubation in the case of glucoraphanin (Figure 2).

Studies with Isothiocyanates. All three isothiocyanates increased quinone reductase activity when incubated for 6 h or longer, which was accompanied by a rise in protein expression with maximum increase in expression being evident at the 24 h incubation point (Figure 3). When the glutathione *S*-transferase activity was monitored using CDNB as a probe substrate, incubation of liver slices with phenethyl isothiocyanate led to a significant rise in activity following incubation periods of 6 or 24 h. However, in the case of erucin, a modest, statistically significant rise in activity was noted following a 4 h incubation, with the effect being far more pronounced following 6 and 24 h incubations (Figure 4). Finally, sulforaphane elicited the most rapid rise in glutathione *S*-transferase activity, with a statistically significant increase in activity being apparent as early as 2 h. When the levels of GSTα were determined immunologically, a gradual rise in expression, observable after only 1 h of exposure, was seen for both erucin and sulforaphane, whereas in the case of phenethyl isothiocyanate, a rise was only evident following 6 and 24 h of exposure.

DISCUSSION

In studies conducted nearly four decades ago, a relationship was observed between the biological half-life of a compound and the potency of induction of cytochrome P450 enzymes. Using a series of barbiturates as model compounds, a direct relationship was established between biological half-life and potency of induction of cytochrome P450 activities, such that phenobarbital, characterized by the longest half-life, was the most effective inducer of these enzymes.[19,20] Clearly, the length of time a compound resides in the body intact is an important determinant of the extent of induction.

A major mechanism of action of chemopreventive isothiocyanates is up-regulation of detoxification enzyme systems such as glutathione *S*-transferases and quinone reductase.[21,22] Recent studies have also indicated that glucosinolates, the precursors of isothiocyanates, not only can be absorbed following oral intake, at least in rats and dogs,[23,24] but also possess biological activity that can contribute to the modulation of such detoxifying enzyme systems.[12,25,26] As most work concerned with this mechanism, as well as other mechanisms of chemoprevention such as cell cycle arrest and induction of apoptosis, has been carried out largely in vitro, the relevance of such studies can only be evaluated in association with achievable plasma/tissue levels. Almost always, the in vitro effects are related to the C_{max}, the highest plasma concentration achieved following dietary levels of intake. In doing so, however, the assumption is made that even a transient attainment of the C_{max} is sufficient to provoke a complete and sustained up-regulation of the enzyme systems.

The purpose of this study was to test this hypothesis using glucosinolates/isothiocyanates, incubating them for different time periods with precision-cut tissue slices, an in vitro system that lends itself to such type of investigations. The glucosinolate/isothiocyanate concentrations were chosen on the basis of our previous studies, are at levels known to elicit marked induction of the relevant enzyme systems,[12,21,22] and can also be achieved by normal levels of intake. In the case of phenethyl isothiocyanate (1 μM), administration to rats of a single oral dose, equivalent to the human dietary intake, achieves such a plasma concentration.[22] In the case of sulforaphane (10 μM), a single modest intake of only 100 g of broccoli soup achieved a plasma concentration of about 2 μM in volunteers.[27] Bearing in mind that the clearance of sulforaphane in rats is about four times faster than in humans,[28,29] the concentrations used in the present study are physiological. Morever, one would expect that plasma levels increase on repeated intake or in individuals consuming glucosinolate supplements. In the case of erucin (10 μM), no pharmacokinetic studies have, to our knowledge, been published. However, erucin is structurally very closely related to sulforaphane. Finally, intact glucosinolates have been detected in the plasma of rat and dogs, but no pharmacokinetic studies have been carried out. No information has been published on plasma concentrations of glucosinolates in humans.

For glucosinolates to induce significantly the dealkylation of the two alkoxyresorufins, used as probes for the Ah-regulated CYP1 family,[30,31] it was essential for the slices to be in contact with the inducing agent for at least 6 h; the rise in CYP1A1 and CYP1A2 apoprotein levels observed in the pooled slices indicates that increased enzyme availability is at least partly responsible for this effect. Although erucin and *R*-sulforaphane, the isothiocyanates emanating from glucoerucin and glucoraphanin, respectively, also up-regulate the CYP1 family at the protein level, no increase in activity can be discerned as these compounds are also effective mechanism-based inhibitors,[32] and for this reason, the dealkylation of alkoxyresorufins was not assessed in the current studies following incubation with the isothiocyanates.

When the isothiocyanates were incubated with precision-cut liver slices and quinone reductase activity was monitored, a similar picture emerged as to the time required for an increase in activity to be manifested. All three isothiocyanates up-regulated quinone reductase activity in rat liver slices, and in all

cases, a minimum exposure of 6 h was essential. However, it should be noted that at the protein level, the aliphatic isothiocyanates gave rise to a very modest rise in expression at earlier time points. Glutathione *S*-transferase activity was monitored using CDNB, a nonspecific substrate whose conjugation with glutathione is catalyzed by a number of glutathione *S*-transferases isoenzymes.[33] As in previous studies,[21,22] the activity of this enzyme was elevated when slices were incubated with any of the three isothiocyanates, but the incubation time required for the rise in activity to be manifested varied with the isothiocyanate, ranging from 2 h in the case of sulforaphane to 6 h in the case of phenethyl isothiocyanate; clearly, the nature of the side chain is important in the up-regulation of this enzyme by isothiocyanates. The decreased exposure period required to elicit an increase in glutathione *S*-transferase activity as compared to other xenobiotic-metabolizing enzymes studied herein may reflect the fact that, in contrast to the other enzymes studied, which are regulated by the Ah receptor, the glutathione *S*-transferase subunits are subject to regulation by other transcription factors.[34] Bearing in mind that sulforaphane has been demonstrated to be an effective antagonist of PXR,[35] a nuclear receptor involved in transcriptional regulation of several glutathione *S*-transferase family members, it is unlikely that PXR mediates the observed effects. However, glutathione *S*-transferase is also under control of the Nrf2 transcription factor that acts as a sensor of cellular stress.[36] It is, hence, tempting to speculate that this early induction of GST expression is mediated via Nrf2, either through direct agonism of the receptor itself or indirectly through depletion of glutathione pools due to rapid conjugation with sulforaphane.[37] The levels of GSTa, one of the classes contributing to the metabolism of CDNB,[38] were also elevated by the three isothiocyanates implying increased enzyme concentration.

What these studies clearly indicate is that a transient attainment of the biologically active concentration of glucosinolates/isothiocyanates in a tissue is not sufficient to lead to enhanced enzyme activity, but longer exposure may be indispensable. A likely contributory mechanism may be related to the fact that isothiocyanates attain very high intracellular concentrations as a result of their interaction with glutathione.[39−42] It has been demonstrated in in vitro studies that peak intracellular concentrations of isothiocyanates are attained within 3 h of exposure, and intracellular concentration may be as much as 200-fold higher than extracellular concentration.[42] Importantly, the ratio of parent compound to the glutathione metabolite changes dramatically during this period; after 30 min of exposure, 95% of the intracellular sulforaphane is present as the glutathione conjugate, whereas after 3 h, only approximately 5% remains in this form.[37] It is thus feasible that the temporal nature of the enzyme induction observed is a consequence of these fluctuating ratios; at early time points, production of glutathione conjugates and depletion of the glutathione pool drive enzyme induction through the Nrf2 transcription factor, whereas at later time points, the increased concentration of free parent compounds allows sufficient activation of the Ah receptor to mediate increases in enzyme systems such as quinone reductase. Indeed, the potential of various isothiocyanates to induce quinone reductase and glutathione *S*-transferase activities was correlated to their intracellular concentration of the isothiocyanate and its dithiocarbamate metabolites.[39,40] Phenethyl isothiocyanate, in contrast to sulforaphane and erucin, failed to up-regulate glutathione *S*-transferase activity following short incubation times and necessitated incubation for at least 6 h. Most likely, this is due to the fact that first phenethyl isothiocyanate penetrates the cell more slowly than sulforaphane, and more importantly, the intracellular levels are maintained for at least 12 h,[39] implying poor metabolism by glutathione conjugation, and second, the inducing concentration of phenethyl isothiocyanate, through the Ah receptor, is much lower than that of erucin and sulforaphane, being 1 and 10 μM, respectively; as a result, no glutathione depletion occurs to drive induction of this enzyme via the Nrf2 transcription factor. It is relevant to point out that in pharmacokinetic studies involving sulforaphane conducted in rats following intravenous and oral dosing and human volunteers following consumption of broccoli, a rapid marked drop was observed in the plasma concentrations of sulforaphane probably representing intracellular accumulation,[28,29] whereas no such effect was observed in the case of phenethyl isothiocyanate following oral administration to rats.[43]

In addition to the requirement for the inducing compound to be present at sufficient concentrations to activate the Ah receptor, it also may be argued that a sustained signaling through this transcription factor may be required for the active protein to be synthesized, which would necessitate a prolonged association of the glucosinolate/isothiocyanate with the appropriate receptor. In support for such a mechanism is the observation that generally the activity/expression of the enzymes increased when the incubation exposure period was increased from 6 to 24 h. Finally, the possibility that small increases in activity occurred at earlier time points but could not be detected because of lack of sensitivity of the enzyme assays can not be ruled out.

In conclusion, the current studies concerned with the temporal induction of cytochrome P450 and phase II enzymes by glucosinolates and isothioycanates, respectively, have established that for an effective rise in the activity of these enzymes to be manifested, tissue exposure of at least 6 h to the appropriate inducing concentrations would be required.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) 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.

(2) Epplein, M.; Wilkens, L. R.; Tiirikainen, M.; Dyba, M.; Chung, F.-L.; Coodman, M. T.; Murphy, S. P.; Henderson, B. E.; Kolonel, L. N.; Le Marchand, L. Urinary isothiocyanates; glutathione *S*-transferase

M1, TI, and *P1* polymorphisms; and risk of colorectal cancer: The multiethnic cohort study. *Cancer Epidemiol. Biomarkers Prev.* **2008**, *18*, 314−320.
(3) Lam, T. K.; Gallichio, L.; Lindsley, K.; Shiels, M.; Hammond, E.; Tao, X.; Chen, L.; Robinson, K. A.; Caulfield, L. E.; Heman, J. G.; Guallar, E.; Alberg, A. J. Cruciferous vegetable consumption and lung cancer risk: A systematic review. *Cancer Epidemiol. Biomarkers Prev.* **2009**, *18*, 184−195.
(4) Steinbrecher, A.; Rohrmann, S.; Timofeeva, M.; Risch, A.; Jansen, E.; Linseisen, J. Dietary glucosinolate intake, polymorphisms in biotransformation enzymes, and risk of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* **2010**, *19*, 135−143.
(5) Steinbrecher, A.; Linseisen, L. Dietary intake of individual glucosinolates in participants of the EIPC-Heidelberg cohort study. *Ann. Nutr. Metab.* **2009**, *54*, 87−96.
(6) Verkerk, R.; Schreiner, M.; Krumbein, A.; Ciska, E.; Holst, B.; Rowland, I.; De Schrijver, R.; Hansen, M.; Gerhäuser, C.; Mithen, R.; Dekker, M. Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. *Mol. Nutr. Food Res.* **2009**, *53*, S219−S265.
(7) Dingley, K. H.; Ubick, E. A.; Chiarappa-Zucca, M. L.; Nowell, S.; Abel, S.; Ebeler, S. E.; Mitchell, A. E.; Burns, S. A.; Steinberg, F. M.; Clifford, A. J. Effect of dietary constituents with chemopreventive potential on adduct formation of a low dose of the heterocyclic amines PhIP and IQ and phase II enzymes. *Nutr. Cancer* **2003**, *46*, 212−221.
(8) Singletary, K.; MacDonald, C. Inhibition of benzo[*a*]pyrene and 1,6-dinitropyrene-DNA adduct formation in mammary epithelial cells by dibenzoylmethatne and sulforaphane. *Cancer Lett.* **2000**, *155*, 47−54.
(9) Bacon, J. T.; Williamson, G.; Garner, R. C.; Lappin, G.; Langouët, S.; Bao, Y. Sulforaphane and quercetin modulate PhIP-DNA adduct formation in human HepG2 cells and hepatocytes. *Carcinogenesis* **2003**, *24*, 1909−1911.
(10) Fimognari, C.; Hrelia, P. Sulforaphane as a promising molecule for fighting cancer. *Mutat. Res.* **2007**, *635*, 90−104.
(11) Traka, M.; Mithen, R. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.* **2009**, *8*, 269−282.
(12) Abdull Razis, A. F.; Bagatta, M.; De Nicola, G. R.; Iori, R.; Ioannides, C. Intact glucosinolates modulate hepatic cytochrome P450 and phase II conjugation activities and may contribute directly to the chemopreventive activity of cruciferous vegetables. *Toxicology* **2010**, *277*, 74−85.
(13) Hashemi, E.; Dobrota, M.; Till, C.; Ioannides, C. Structural and functional integrity of precision-cut liver slices in xenobiotic metabolism: A comparison of the dynamic organ and multiwell plate culture procedures. *Xenobiotica* **1999**, *29*, 11−25.
(14) 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.
(15) 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.
(16) Prohaska, HJ.; 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.
(17) 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.
(18) 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**, *72*, 248−254.
(19) Valerino, D. M.; Vesell, E. S.; Aurori, K. C.; Johnson, A. O. Effect of various barbiturates on hepatic microsomal enzymes. A comparative study. *Drug Metab. Dispos.* **1974**, *2*, 448−457.
(20) Ioannides, C.; Parke, D. V. Mechanism of induction of hepatic microsomal drug metabolizing enzymes by a series of barbiturates. *J. Pharm. Pharmacol.* **1975**, *27*, 739−746.
(21) Hanlon, N.; Poynton, C. L.; Coldham, N.; Sauer, M. J.; Ioannides, C. The aliphatic isothiocyanates erucin and sulforaphane do not effectively up-regulate NAD(P)H: quinone oxidoreductase (NQO1) in human liver compared with rat. *Mol. Nutr. Food Res.* **2009**, *53*, 836−844.
(22) Konsue, N.; Ioannides, C. Differential response of four human livers to modulation of phase II enzyme systems by the chemopreventive phytochemical phenethyl isothiocyanate. *Mol. Nutr. Food Res.* **2010**, *54*, 426−432.
(23) Cwik, M. J.; Wu, H.; Muzzio, M.; McCormick, D. L.; Kapetanovic, I. Direct quantitation of glucoraphanin in dog and rat plasma by LC-MS/MS. *J. Pharm. Biomed. Anal.* **2010**, *52*, 544−549.
(24) Bheemreddy, R. M.; Jeffery, E. H. The metabolic fate of purified glucoraphanin in F344 rats. *J. Agric. Food Chem.* **2007**, *55*, 2861−2866.
(25) Abdull Razis, A. F.; Bagatta, M.; De Nicola, G. R.; Iori, R.; Ioannides, C. Induction of epoxide hydrolase and glucuronosyl transferase by isothiocyanates and intact glucosinolates in precision-cut liver slices: Important of side chain substituent and chirality. *Arch. Toxicol.* **2011**, *85*, 919−927.
(26) Abdull Razis, A. F.; Bagatta, M.; De Nicola, G. R.; Iori, R.; Ioannides, C. Up-regulation of cytochrome P450 and Phase II enzyme systems in rat precision-cut rat lung slices by the intact glucosinolates, glucoraphanin and glucoerucin. *Lung Cancer* **2011**, *71*, 298−305.
(27) Gasper, A. V.; Al-janobi, A.; Smith, J. A.; Bacon, J. R.; Fortun, P.; Atherton, C.; Taylor, M. A.; Hawkey, C. J.; Barrett, D. A.; Mithen, R. F. Glutahtione *S*-transferase M1 polymorphism from standard and high-glucosinolate broccoli. *Am. J. Clin. Nutr.* **2005**, *82*, 1283−1291.
(28) 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 the rat. *Br. J. Nutr.* **2008**, *99*, 559−564.
(29) Hanlon, N.; Coldham, N.; Gielbert, A.; Kuhnert, N.; Sauer, M. J.; Ioannides, C. Repeated intake of broccoli does not lead to higher plasma levels of sulforaphane in human volunteers. *Cancer Lett.* **2009**, *284*, 15−20.
(30) Namkung, M. J.; Yang, H. L.; Hulla, J. E.; Juchau, M. R. On the substrate specificity of cytochrome P450IIIA1. *Mol. Pharmacol.* **1988**, *34*, 628−637.
(31) Lum, P. Y.; Burke, M. D.; Mayer, R. T.; Ioannides, C Alkoxyresorufin O-dealkylases: association with the murine Ah locus. *Cancer Lett.* **1986**, *32*, 255−262.
(32) Hanlon, N.; Okpara, M.; Coldham, N.; Sauer, M. J.; Ioannides, C. Modulation of rat hepatic and pulmonary cytochromes P450 and Phase II enzyme systems by erucin, an isothiocyanate structurally related to sulforaphane. *J. Agric. Food Chem.* **2008**, *56*, 7866−7871.
(33) 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.
(34) Higgins, L. G.; Hayes, J. D. Mechanisms of induction of cytosolic and microsomal glutathione transferase (*GST*) genes by xenobiotics and pro-inflammatory agents. *Drug Metab. Dispos.* **2011**, *43*, 92−137.
(35) Zhou, C.; Poulton, E.-J.; Grun, F.; Bammler, T. K.; Blumberg, B.; Thummel, K. E.; Eaton, D. L. The dietary isothiocyanate sulforaphane is an antagonist of the human steroid and xenobiotic nuclear receptor. *Mol. Pharmacol.* **2007**, *71*, 220−229.
(36) McMahon, M.; Itoh, K.; Yamamoto, M.; Chanas, S. A.; Henderson, C. J.; McLellan, L. I.; Wolf, C. R.; Cavin, C.; Hayes, J. D. The cap 'n' cooar basic leucine zipper transcription factor Nrf2 (NF-E2p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res.* **2001**, *61*, 3299−3307.

(37) Zhang, Y. S. Role of glutathione in the accumulation of anticarcinogenic isothiocyanates and their glutathione conjugates by murine hepatoma cells. *Carcinogenesis* **2000**, *21*, 1175−1182.
(38) Sherratt, P. J.; Hayes, J. D. Glutathione *S*-transferases. In *Enzyme Systems that Metabolise Drugs and other Xenobiotics*; Ioannides, C., Ed.; John Wiley & Sons, Ltd.: Chichester, United Kingdom, 2002; pp 319−352.
(39) Zhang, Y.; Talalay, P. Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. *Cancer Res.* **1998**, *58*, 4632−4639.
(40) Ye, L.; Zhang, Y. Total intracellular accumulation levels of dietary isothiocyanates determine the activity in elevation of cellular glutathione and phase 2 detoxication enzymes. *Carcinogenesis* **2001**, *22*, 1987−1992.
(41) Zhang, Y.; Callaway, E. C. High cellular accumulation of sulforaphane, a dietary anticarcinogen, is followed by rapid transporter-mediated export as a glutathione conjugate. *Biochem. J.* **2002**, *364*, 301−307.
(42) Zhang, Y. Cancer-preventive isothiocyanates: Measurement of human exposure and mechanism of action. *Mutat. Res.* **2004**, *555*, 173−190.
(43) Konsue, N.; Kirkpatrick, J.; Kuhnert, N.; King, L. J.; Ioannides, C. Repeated oral administration modulates the pharmacokinetic behaviour of the chemopreventive agent phenethyl isothiocyanate in rats. *Mol. Nutr. Food Res.* **2010**, *54*, 426−432.