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ORIGINAL CONTRIBUTION

A glucosinolate-rich extract of Japanese Daikon perturbs carcinogen-metabolizing enzyme systems in rat, being a potent inducer of hepatic glutathione *S*-transferase

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Abstract
Purpose Glucosinolates/isothiocyanates are an established class of naturally occurring chemopreventive agents, a principal mechanism of action being to limit the generation of genotoxic metabolites of chemical carcinogens, as a result of modulation of cytochrome P450 and phase II detoxification enzymes. The objective of this study was to assess whether a glucosinolate-rich extract from Daikon sprouts, containing glucroraphasatin and glucoraphenin, is a potential chemopreventive agent by modulating such enzymes in the liver and lung of rats.
Methods Rats were exposed to the glucosinolate-rich Daikon extract through the diet, at three dose levels, for 14 days, so that the low dose simulates dietary intake.
Results At the low dose only, a modest increase was noted in the hepatic dealkylations of methoxy-, ethoxy-, pentoxyresorufin and benzyloxyquinoline that was accompanied by elevated expression of CYP1 and CYP3A2 apoprotein levels. In lung, only a modest increase in the dealkylation of pentoxyresorufin was observed. At higher doses, in both tissues, these increases were abolished. At the same low dietary dose, the Daikon extract elevated markedly glutathione *S*-transferase activity paralleled by rises in GSTα, GSTμ and GSTπ protein expression. An increase was also noted in quinone reductase activity and expression. Finally, glucuronosyl transferase and epoxide hydrolase activities and expression were also up-regulated, but necessitated higher doses.
Conclusion Considering the ability of Daikon glucosinolates to effectively enhance detoxification enzymes, in particular glutathione *S*-transferase, it may be inferred that consumption of this vegetable may possess significant chemopreventive activity and warrants further evaluation through epidemiology and studies in animal models of cancer.

Keywords Daikon · Glucosinolates · Glucoraphasatin · Isothiocyanates · Chemoprevention · Glutathione *S*-transferase

Introduction

A number of mechanisms may contribute to the established chemopreventive activity of glucosinolates and of their degradation products, the isothiocyanates, one of the principals being favourable modulation of the enzyme systems involved in the metabolism of chemical carcinogens [1]. As a result, the generation of reactive intermediates that interact with DNA is impaired. Indeed, the aliphatic isothiocyanate sulforaphane suppressed the DNA binding of the polycyclic aromatic hydrocarbons benzo[a]pyrene and 1,6-dinitropyrene in human mammary epithelial cells [2], and the formation of DNA adducts with the heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in human hepatocytes [3]. The aromatic isothiocyanate phenethyl isothiocyanate

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similarly attenuated the formation of DNA adducts in rats treated with PhIP [4]. Moreover, phenethyl isothiocyanate antagonized the carcinogenicity of the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in rat and inhibited DNA-adduct formation in the lung [5]. The same isothiocyanates decreased the formation of benzo[a]-pyrene-induced DNA adducts in A/J mice [6]. Pre-treatment of rats with another aromatic isothiocyanate, benzyl isothiocyanate, caused a marked reduction in heterocyclic amine-induced DNA damage in colon and liver cells [7]. Finally, Boysen et al. [8] observed a reduction in pulmonary DNA adducts in rats treated with benzo[a]pyrene through the diet and NNK in drinking water, and either phenethyl isothiocyanate or a mixture of phenethyl isothiocyanate and benzyl isothiocyanate in the diet. Clearly, isothiocyanates can impede the formation of DNA adducts following exposure to chemical carcinogens.

A mechanism through which isothiocyanates can limit the generation of reactive intermediates is via inhibition of their cytochrome P450 generation, the most active family being CYP1 [9]. In in vivo studies conducted in rats, administration of phenethyl isothiocyanate [10] or sulforaphane [11] decreased CYP1 activity in the liver even following the intake of dietary doses. Indeed, isothiocyanates are mechanism-based inhibitors of cytochrome P450 [10–12]. However, in studies performed in precision-cut rat liver slices, isothiocyanates proved to be also effective inducers of phase II enzyme systems including the glutathione *S*-transferases, glucuronosyl transferase, epoxide hydrolase (EH) and quinone reductase that lead to detoxification of the genotoxic metabolites of chemical carcinogens [13–15]. Thus, isothiocyanates have the potential to limit the availability of the DNA-interacting intermediates by preventing their formation and, in particular, stimulating their elimination.

Glucoraphasatin (4-methylsulfanyl-3-butenyl glucosinolate, glucodehydroerucin, CAS number 28463-23-2) is a glucosinolate whose only important source is *Raphanus sativus* (Kaiware Daikon), a white radish that is very extensively consumed in Japan and increasingly in Europe and North America [16]. When it comes into contact with myrosinase (*β*-thioglucoside glucohydrolase, EC.3.2.1.147), glucoraphasatin is converted to 4-methylsulfanyl-3-butenyl isothiocyanate (raphasatin). Both glucoraphasatin and raphasatin have the potential to enhance phase II activities as illustrated in HepG2 cells and precision-cut rat liver slices, being especially potent inducers of glutathione *S*-transferase [17–19]. These observations prompted us to carry out the first in vivo study in rat to assess the potential of Daikon glucosinolates, the major being glucoraphasatin, to perturb enzyme systems, involved in the generation and detoxification of genotoxic metabolites of chemical carcinogens, in the liver and lung following the exposure of rats to low doses, commensurate with human levels of dietary intake.

Materials and methods

Ethoxyresorufin, methoxyresorufin, pentoxyresorufin, resorufin, *β*-naphthol, 3′-phosphoadenosine-5′-phosphpsulphate, 1-naphthol, 4-aminobenzoic acid, acetyl-CoA, NADPH, 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), cytochrome c peroxidase-linked anti-rabbit, anti-mouse and anti-goat antibodies (Sigma-Aldrich Ltd., Poole, Dorset, UK), benzo[a]pyrene 4,5-epoxide and benzo[a]pyrene 4,5-diol (MidWest Research Institute, Kansas, USA), chloro-4-nitrobenzo-2-oxa-1,3-diazole (Fluka, Buchs SG, Switzerland), and anti-CYP1A1 (AMS Biotechnology, Abingdon, UK), anti-CYP1A2 (Abcam, Cambridge, UK) and anti-CYP1B1 (BD Biochemicals, Oxford, UK) antibodies were all purchased. Antibodies to rat CYP3A, human quinone reductase, lactate dehydrogenase and *β*-actin were obtained from Abcam (Cambridge UK), antibody to GSTα from Calbiochem (Lutterworth, UK) and antibodies to EH, glucuronosyl transferase (UGT1A6), sulphotransferase, *N*-acetyltransferase (NAT-1/2) as well as donkey anti-goat and goat anti-rabbit antibodies from Santa Cruz Biotechnology (California, USA). The preparation of the glucosinolate-rich extract from 7-day-old freeze-dried Japanese Daikon sprouts (Kaiware Daikon) has already been described [18]. Two glucosinolates are present in this extract, glucoraphasatin (253 ± 9 μmol/g; 11.6 ± 0.4 % w/w) and glucoraphenin (116 ± 10 μmol/g; 5.5 ± 0.5 % w/w). Very low levels of indole glucosinolates are also present (0.5 ± 0.2 % w/w).

Animal treatment

The study was conducted according to the Animals (Scientific Procedures) Act 1986. Male Wistar albino rats (180 ± 20 g) were obtained from Charles River UK Ltd (Margate, Kent, UK). 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. They were randomly divided into four groups, each comprising five animals. Since the human intake of glucoraphasatin and glucoraphenin, to our knowledge, has not been reported, the doses employed in the present study were based on the average human consumption of total glucosinolates in the UK, which is 14 mg/person/day [20]; assuming an average body weight of 70 kg, this is equivalent to 0.2 mg/kg/day. Diets were supplemented with the glucosinolate extract, so that the daily doses of total glucosinolate were approximately 0.5 (Low), 5.0 (Medium) and 50 (High) mg/kg/day, whereas the 4th group served as control. The animals were maintained on these diets for 14 days, and body weights were recorded daily. At the end of this period, rats were killed by cervical dislocation, tissues were immediately excised and post-mitochondrial fractions prepared and stored at −80 °C until use. When required, samples were

thawed and resolved to microsomal and cytosolic fractions by centrifugation (105,000$g \times 1$ h).

Enzyme assays

The following assays were carried out on isolated microsomes: the dealkylations of methoxyresorufin [21], ethoxyresorufin [22], pentoxyresorufin [23] and 7-benzyloxyquinoline [24], glucuronosyl transferase (UDP-GT) using 1-naphthol as substrate [25] and EH using benzo[a]pyrene 4,5-epoxide [26]. The following determinations were carried out on the cytosolic fraction: quinone reductase (NQO1) using menadione as substrate [27], glutathione *S*-transferase activity [28] monitored using CDNB, DCNB and chloro-4-nitrobenzo-2-oxa-1,3-diazole as accepting substrates, *N*-acetyltransferase using 4-aminobenzoic as substrate [29], sulphotransferase using β-naphthol as substrate [30] and total glutathione levels [31]. Protein concentration was determined in both cellular subfractions using bovine serum albumin as standard [32]. Finally, in order to monitor changes in enzyme protein expression to assess whether changes in activity are reflected by alterations in enzyme expression, Western blot analysis was performed using pooled microsomes/cytosol from five animals. Hepatic or pulmonary microsomal or cytosolic proteins from pooled animals were loaded on to 10 % (w/v) SDS-PAGE (sodium dodecyl phosphate polyacrylamide gel electrophoresis), and then transferred electrophoretically to Hybond-P polyvinylidene difluoride membrane. The immunoblot analysis of rat proteins was carried out by exposure to the primary antibody followed by the appropriate peroxidase-labelled secondary antibody. β-actin and lactate dehydrogenase were used as the housekeeping proteins to normalize protein loading for microsomal and cytosolic proteins, respectively. Molecular markers were run concurrently. Immunoblots were quantitated by densitometry using the GeneTool software (Syngene Corporation, Cambridge, UK), with the control band designated as 100 %.

Statistical evaluation

Results are presented as mean $\pm$ standard deviation of five animals. Statistical evaluation was carried out by one-way ANOVA followed by the Dunnett's test.

Results

None of the treatments had any effect on animal body weight gain (results not shown). Treatment of rats with the low dose of the Daikon extract led to a statistically significant rise in the *O*-dealkylation of the three alkoxyresorufin substrates as well as of benzyloxyquinoline (Table 1). However, no such increase was observed at the higher doses; in the case of ethoxyresorufin, activity was impaired at the two higher doses. A very similar picture emerged in the expression of CYP1A1/A2/B1 and CYP3A2 apoprotein levels determined immunologically by Western blotting (results not shown). In contrast to the liver, treatment at any dose failed to modulate the *O*-deethylation of ethoxyresorufin in the lung, whereas the *O*-dependentylation of pentoxyresorufin was increased, but only at the Low dose (Table 2). At the protein level, pulmonary CYP1A1 expression was not influenced by glucoraphasatin, whereas CYP1B1 and CYP3A2 were clearly increased, but only at the low dose (results not shown).

Hepatic glutathione *S*-transferase activity was monitored using three substrates; whatever the substrate, exposure to the Daikon extract gave rise to a significant increase in activity, which was particulary marked in the case of CDNB (Table 1). Enhanced activity could be seen even at the low level of intake of the glucosinolate extract. Commensurate with these observations, a clear rise in the expression of GSTα and, to a lesser extent, GSTμ and GSTπ was noted (results not shown). Glucuronosyl transferase as well as EH activities were also up-regulated in the liver of rats, but only at the highest dose of the extract, whereas no such increase was noted in sulphotransferase or *N*-acetyltransferase activities (Table 1). Immunoblot analysis revealed elevated expression in the case of glucuronosyl transferase and EH, but not sulphotransferase or *N*-acetyltransferase in concordance with the activity changes (results not shown). Quinone reductase activity and expression were elevated in the liver of rats fed the low and medium Daikon extract diets (Table 1); finally, no change in total glutathione levels was manifested at all dose levels (Table 1).

A completely different picture was noted when the same phase II activities were determined in the lung. None of the enzymes studied, at any dose level, was modulated by the intake of glucoraphasatin (Table 2). Similarly, at the protein level, no changes were observed in the levels of GSTα, GSTπ, GSTμ, quinone reductase, sulphotransferase, *N*-acetyltransferase, glucuronosyl transferase or EH (results not shown).

Discussion

The realization that cruciferous vegetables possess chemopreventive activity led to further studies to identify the components responsible and to elucidate the underpinning mechanism(s). As glucosinolates and their enzymically generated metabolites, the isothiocyanates, are present in these vegetables at substantial amounts, attention focussed on these chemicals as the likely constituents responsible for the chemopreventive activity of cruciferous vegetable. In

Table 1 Hepatic carcinogen-metabolizing hepatic enzyme activities in rats fed a glucosinolate-rich Daikon extract

Enzyme activity	Daikon extract dose			
	Control	Low dose	Medium dose	High dose
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)	42.1 ± 4.9	56.4 ± 7.5**	34.6 ± 1.2*	26.3 ± 5.0***
Methoxyresorufin *O*-demethylase (pmol/min/mg protein)	32.8 ± 4.8	50.8 ± 3.1***	31.3 ± 7.4	28.9 ± 5.6
Pentoxyresorufin *O*-depentylase (pmol/min/mg protein)	11.4 ± 1.9	14.2 ± 1.9*	11.4 ± 1.7	9.3 ± 1.0
7-Benzoyloxyquinoline dealkylation (pmol/min/mg protein)	160 ± 29	266 ± 72*	156 ± 33	125 ± 43
Quinone reductase (nmol/min/mg protein)	95 ± 7	136 ± 21**	123 ± 22*	99 ± 9
Glutathione *S*-transferase (CDNB) (μmol/min/mg protein)	0.18 ± 0.01	1.83 ± 0.10***	1.99 ± 0.14***	0.24 ± 0.02***
Glutathione *S*-transferase (DCNB) (μmol/min/mg protein)	0.15 ± 0.02	0.20 ± 0.03**	0.20 ± 0.02**	0.20 ± 0.02**
Glutathione *S*-transferase (chloro-4-nitrobenzo-2-oxa-1,3-diazole) (μmol/min/mg protein)	0.23 ± 0.04	0.27 ± 0.06	0.29 ± 0.02*	0.34 ± 0.02***
Sulphotransferase (μmol/min/mg protein)	8.21 ± 1.92	8.33 ± 1.97	6.11 ± 0.61*	7.15 ± 1.21
N-Acetyltransferase (nmol/min/mg protein)	15.2 ± 3.1	14.4 ± 3.9	11.0 ± 0.9*	12.5 ± 2.2
Epoxide hydrolase (nmol/min/mg protein)	1.25 ± 0.18	1.22 ± 0.19	1.33 ± 0.30	2.06 ± 0.14***
Glucuronosyl transferase (nmol/min/mg protein)	108 ± 13	116 ± 13	125 ± 19	230 ± 26***
Total glutathione (mM)	3.69 ± 0.95	3.54 ± 0.41	3.17 ± 0.59	3.17 ± 0.37

Groups of five rats were fed diets supplemented with a glucosinolate-rich Daikon extract at a total glucosinolate dose of 0.5 (Low dose), 5.0 (Medium dose) and 50 (High dose) mg/kg/day for 14 days, whereas one group served as control. At the end of the treatment period, rats were killed and livers were removed. Results are presented as mean ± SD of five rats. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Pulmonary carcinogen-metabolizing enzyme activities in rats fed a glucosinolate-rich Daikon extract

Enzyme activity	Daikon extract dose			
	Control	Low dose	Medium dose	High dose
Ethoxyresorufin *O*-deethylase (pmol/min/mg protein)	30.6 ± 6.1	28.4 ± 3.4	29.1 ± 4.8	24.1 ± 3.0
Pentoxyresorufin *O*-depentylase (pmol/min/mg protein)	8.44 ± 1.05	12.5 ± 2.9*	9.00 ± 1.64	8.03 ± 1.57
Quinone reductase (nmol/min/mg protein)	71 ± 9	79 ± 8	81 ± 5	75 ± 8
Glutathione *S*-transferase (CDNB) (μmol/min/mg protein)	0.18 ± 0.04	0.17 ± 0.02	0.16 ± 0.03	0.16 ± 0.04
Glutathione *S*-transferase (chloro-4-nitrobenzo-2-oxa-1,3-diazole) (μmol/min/mg protein)	0.24 ± 0.03	0.22 ± 0.04	0.25 ± 0.04	0.19 ± 0.04
Sulphotransferase (μmol/min/mg protein)	6.51 ± 1.02	6.32 ± 1.07	5.74 ± 0.98	5.98 ± 1.61
N-Acetyltransferase (nmol/min/mg protein)	11.6 ± 1.4	11.1 ± 1.5	10.6 ± 2.2	9.9 ± 2.5
Epoxide hydrolase (pmol/min/mg protein)	66.5 ± 7.3	68.0 ± 8.75	63.4 ± 5.6	62.2 ± 2.9
Glucuronosyl transferase (nmol/min/mg protein)	94 ± 8	103 ± 7	106 ± 12	108 ± 16
Total glutathione (mM)	0.81 ± 0.20	0.72 ± 0.22	0.74 ± 0.31	0.72 ± 0.25

Groups of five rats were fed diets supplemented with a glucosinolate-rich Daikon extract at a total glucosinolate dose of 0.5 (Low dose), 5.0 (Medium dose) and 50 (High dose) mg/kg/day for 14 days, whereas one group served as control. Results are presented as mean ± SD of five rats. * $P < 0.05$

laboratory studies conducted in animal models of cancer, isothiocyanates proved to be effective in protecting against chemical carcinogens [33, 34]. Studies aimed at unravelling the mechanisms of action revealed that cruciferous vegetables impaired the ability of carcinogenic compounds to form DNA adducts. Brussels sprouts and garden cress juices have been shown to suppress IQ-induced DNA damage, and preneoplastic lesions in the colon and liver of rats treated with 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ) [35]. Moreover, aqueous extracts of Brussels sprouts decreased DNA damage induced by 2-nitropropane [36], whereas supplementation of the diet with freeze-dried Brussels sprouts impaired IQ-induced DNA damage in the liver of rats [37]. Garden cress inhibited the IQ-induced genotoxicity and the appearance of preneoplastic lesions in rat colon [7]. Further studies carried out by the same group demonstrated that garden cress and water cress juices suppressed benzo[a]pyrene-induced DNA damage in HepG2 cells [38]. It is now clear that isothiocyanates, and their precursor glucosinolates, impair the formation of DNA adducts by decreasing their cytochrome P450-mediated generation and, more importantly, by facilitating their

elimination through induction of detoxification enzyme systems such as quinone reductase, EH, glucuronosyl transferase glutathione *S*-transferase; up-regulation of these enzyme systems was observed in both rat lung and liver, although the response of human liver was more variable [13–15, 39, 40].

Glucoraphasatin has been isolated from Daikon, a white radish that is very popular in Japan [41]. In studies emanating from our own laboratory and conducted in vitro using precision-cut liver slices, it was observed that this glucosinolate, as well as its corresponding isothiocyanate, namely raphasatin, up-regulated detoxification enzymes at concentrations as low as 1 μM [18]. These observations prompted us to undertake in vivo studies following the exposure of rats to three doses of a glucosinolate-rich extract from Daikon sprouts, the lowest representing the human dietary dose based on body weight; the principal glucosinolate in this extract is glucroraphasatin, but it also contains substantial amounts of glucoraphenin. At the lowest dietary dose, the Daikon extract up-regulated glutathione *S*-transferase and quinone reductase; the former activity was determined using CDNB, a substrate for a number of the cytosolic transferases, DCNB a substrate associated with the μ-family [42] and finally, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole, which has been reported to be selective for the α-class [43]. The increase in activity, when monitored using CDNB, was exceptional (tenfold), which renders it probably the most potent glucosinolate studied and potentially a strong anti-carcinogen; to our knowledge, the chemopreventive activity of this glucosinolate/isothiocyanate has, so far, not been investigated. Immunoblot analysis was carried out to establish whether the rise in activity involved increased enzyme availability. The increase in activity of the various enzymes was always paralleled by similar changes in enzyme protein levels, indicating enhanced protein expression, most likely the consequence of increased transcription. In the case of glutathione *S*-transferase, the rise in activity was accompanied by increases in the expression of GSTα, GSTμ and, to a lesser extent, GSTπ, indicating that the effect is not isoform specific. Increasing the dose had minimal or no impact on the activity, and expression of these enzymes, and in the case of glutathione *S*-transferase the inductive effect, was suppressed at the highest dose. Whether this represents dose-dependent pharmacokinetic behaviour remains to be established. However, glucuronosyl transferase and EH activity and expression were up-regulated only at the highest dose studied, indicating that phase II detoxification pathways vary in their sensitivity to Daikon glucosinolates. Moreover, up-regulation of the various enzymes may involve different chemical entities, that is, intact glucosinolates, isothiocyanates or isothiocyanate metabolites. It should be pointed out that, at least in the case of raphasatin, its metabolites retain some potential to stimulate carcinogen-metabolizing enzyme systems [19]. In contrast to the observations in the liver, none of the detoxification enzymes was up-regulated in the lung indicating that glucoraphasatin displays tissue specificity. Similar observations have been previously reported for other glucosinolates/isothiocyanates. Supplementation of the diet of rats with phenethyl isothiocyanate caused a rise in glutathione *S*-transferase and quinone reductase activities in the liver, whereas the lung was refractive [10, 44]. Similarly, in in vitro studies employing precision-cut rat tissue slices, glucoerucin and glucoraphanin failed to modulate quinone reductase in the lung [39], whereas in the liver an increase in activity was observed [40]. It is conceivable that the lack of response observed in the lung may reflect poor distribution of the glucosinolates/isothiocyanates in this tissue.

In the present study, the activity of the CYP1 and CYP3 families was also monitored because of the extensive role in the metabolism and bioactivation of chemical carcinogens of the former and the pivotal role of the latter in the metabolism of drugs [9]. Feeding rats with the Daikon extract caused a modest, but statistically significant, rise in the *O*-dealkylations of methoxy-, ethoxy- and pentoxyresorufin, probes for CYP1A2, CYP1A1 and CYP2B, respectively [45], that was accompanied by similar changes in apoprotein expression; the effect, however, was abolished at the higher doses. Although the CYP1 family of cytochrome P450 enzymes is the most active in the bioactivation of chemical carcinogens [9], the increase observed in the present study is very modest compared with polycyclic aromatic hydrocarbons that are also encountered in food, being generated during cooking. Similarly, an increase in CYP3A activity, as exemplified by the dealkylation of benzyloxyquinoline [24], and expression was noted in the liver of rats exposed to the low dose of the Daikon extract. Finally, in the lung, and only at the low dose, an increase in the CYP2B-mediated pentoxyresorufin dealkylation was noted, but the CYP1A1-catalysed deethylation of ethoxyresorufin was unaffected. CYP1A2 is not expressed in the lung, and the dealkylation of benzyloxyquinoline is very low; however, a small increase in CYP3A2 expression in the lung of animals exposed to the low dose was observed. Clearly, the effects of glucoraphasatin on the cytochrome P450 enzyme system are both concentration- and tissue-dependent.

It is not possible to discern whether the observed changes are due to the glucosinolates and/or the generated isothiocyanates. It is now recognized that, at least in the rat or dog, glucosinolates can survive the passage through the intestine to reach the systemic circulation [46, 47]. Although the Daikon extract was administered in the absence of myrosinase, microbial myrosinase present in the

intestine can catalyse the formation of the isothiocyanate [48, 49]. It is pertinent to point out that glucoraphasatin is an excellent substrate of *Sinapis alba* myrosinase, so that exposure to the enzyme at pH 7.4 leads to its complete hydrolysis [50]. The fact that a good biological response was observed even at the lowest level of exposure indicates that the glucosinolates/isothiocyanates are well absorbed and can attain sufficiently high intracellular concentrations to modulate carcinogen-metabolizing enzyme systems.

In conclusion, the present ex vivo studies have established that a glucosinolate-rich Daikon extract, although a modest inducer of hepatic CYP1 and CYP3A, is more effective in up-regulating detoxification enzymes; however, these effects are not manifested in the lung. The ability of Daikon glucosinolates to induce glutathione *S*-transferase activity is potent, indicating that Daikon may be a an effective chemopreventive agent, and further work is warranted through epidemiology and laboratory studies in animal models of cancer.

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