Brassica, Biotransformation and Cancer Risk: Genetic Polymorphisms Alter the Preventive Effects of Cruciferous Vegetables[1]

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ABSTRACT The chemoprotective effect of cruciferous vegetables is due to their high glucosinolate content and the capacity of glucosinolate metabolites, such as isothiocyanates (ITC) and indoles, to modulate biotransformation enzyme systems (e.g., cytochromes P450 and conjugating enzymes). Data from molecular epidemiologic studies suggest that genetic and associated functional variations in biotransformation enzymes, particularly glutathione *S*-transferase (GST)M1 and GSTT1, which metabolize ITC, alter cancer risk in response to cruciferous vegetable exposure. Moreover, genetic polymorphisms in receptors and transcription factors that interact with these compounds may further contribute to variation in response to cruciferous vegetable intake. This review outlines the metabolism and mechanisms of action of cruciferous vegetable constituents, discusses the recent human studies testing effects of cruciferous vegetables on biotransformation systems and summarizes the epidemiologic and experimental evidence for an effect of genetic polymorphisms in these enzymes on response to cruciferous vegetable intake. Taken together, genetic differences in biotransformation enzymes and the factors that regulate them, as well as variation in glucosinolate content of cruciferous vegetables and the methods used to prepare these foods underscore the multiple layers of complexity that affect the study of gene-diet interactions and cancer risk in humans. J. Nutr. 132: 2991–2994, 2002.

KEY WORDS: • isothiocyanates • cruciferae • biotransformation • polymorphism • chemoprevention

Biotransformation enzymes, also referred to as xenobiotic- or drug-metabolizing enzymes, play a major role in regulating the toxic, mutagenic and neoplastic effects of chemical carcinogens, as well as metabolizing other xenobiotics (e.g., phytochemicals and therapeutic drugs) and endogenous compounds (e.g., steroid hormones). Phytochemicals in plant foods modulate biotransformation enzyme activities, one mechanism by which fruits and vegetables, and cruciferous vegetables in particular, may contribute to reduced cancer risk (1).

There are two main groups of biotransformation enzymes. Phase I enzymes (cytochromes P450 and flavin-dependent monooxygenases) convert hydrophobic compounds to reactive electrophiles by oxidation, hydroxylation and reduction reactions to prepare them for reaction with water-soluble moieties. Phase II enzymes (e.g., glutathione *S*-transferases (GST)[3], UDP-glucuronosyltransferases (UGT), sulfotransferases, *N*-acetyltransferases) primarily catalyze conjugation reactions. Genetic polymorphisms in these enzyme systems can influence cancer susceptibility when coupled with the relevant carcinogen exposures; however, only recently have we gained understanding of how genetic differences in components of the biotransformation pathways alter response to chemopreventive foods such as cruciferous vegetables.

In this review we outline the metabolism and mechanisms of action of cruciferous vegetable constituents, discuss the recent human studies testing effects of cruciferous vegetables on biotransformation systems and summarize the epidemiologic and experimental evidence for an effect of genetic polymorphisms in these enzymes on response to cruciferous vegetable intake. We restrict our discussion to work in humans; in vitro and animal model data have been reviewed (2–4).

Glucosinolates and human metabolism

The unique effectiveness of cruciferous vegetables to protect against neoplastic disease is attributed to the fact that they are the richest sources of glucosinolates in the human diet. The family Cruciferae (syn. Brassicaceae) is comprised of familiar foods of the species *Brassica oleracea* (e.g., cabbage, broccoli, cauliflower, Brussels sprouts, kohlrabi and kale) as well as >350 other genera that include a variety of food plants (e.g., arugula, radish, daikon, watercress, horseradish and wasabi) (5). A recent review provides a comprehensive survey of these known glucosinolates and the plant families from which they have been isolated (5).

Glucosinolates (β-thioglycoside-*N*-hydroxysulfates) are hydrolyzed by the plant enzyme myrosinase when the cells in plants are damaged (e.g., cut, ground or chewed), releasing the biologically active isothiocyanates (ITC). If myrosinase has been inactivated (e.g., with cooking), intestinal microbial metabolism of glucosinolates also contributes to ITC exposure, albeit at a lower level (6). Even within the *Brassica* genus and species different glucosinolates predominate and yield distinct ITC (5). For example, glucoraphanin accounts for 35–60% of glucosinolates in broccoli (7) and is converted to the ITC sulforaphane, whereas gluconasturtiin, found in watercress, is hydrolyzed to phenethyl ITC (PEITC). Glucobrassicin in

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[3] Abbreviations: AhR, aryl hydrocarbon receptor; ARE/EpRE; antioxidant/electrophile response element; DIM, diindolylmethane; GST, glutathione *S*-transferase; I3C, indole-3-carbinol; ITC, isothiocyanate; NQO1, NAD(P)H:quinone oxidoreductase; PEITC, phenethyl ITC; UGT, UDP-glucuronosyltransferase; XRE, xenobiotic response element.

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broccoli and Brussels sprouts (8) is broken down to indole-3-carbinol (I3C), which is further converted to a range of polyaromatic indolic metabolites (e.g., diindolylmethane (DIM)) under acid conditions in the stomach. Furthermore, glucosinolate profiles and concentrations not only differ by *Brassica* species but also vary substantially across cultivars and with different growth conditions (9).

The primary route of in vivo metabolism of ITC is by the mercapturic acid pathway, a major pathway for elimination of many xenobiotics (7). Thiol conjugates of ITC are formed by conjugation with glutathione, a reaction catalyzed by GST. Subsequent stepwise cleavage of glutamine and glycine yields L-cysteine-ITC, which are acetylated to produce *N*-acetyl-L-cysteine ITC conjugates (mercapturic acids); these are excreted in urine. Thus, GST play an important role in disposition of ITC in humans. Benzyl ITC, PEITC, allyl ITC, and sulforaphane—common ITC in cruciferous vegetables—are all catalyzed by the four major human GST: GSTA1-1, GSTP1-1, GSTM1-1 and GSTM2-2; however, reaction velocities can differ by as much as 700-fold, and there is wide variation in the extent to which ITC are disposed (10). Thus, in total, human exposure to ITC is influenced by the types and amounts of vegetables consumed, food preparation, how well food is chewed and differences in GST isozyme profiles.

Mechanisms of action of ITC

Compounds in cruciferous vegetables affect biotransformation enzyme activity by several mechanisms. They induce expression of phase I and phase II enzymes and, to a lesser extent, also directly inhibit the P450 (7). The mode of induction by compounds in *Cruciferae* is largely dependent on their structures, with effects of indole derivatives and ITC being distinct (**Fig. 1**). Binding of I3C acid condensates (e.g., DIM) to the aryl hydrocarbon receptor (AhR) leads to translocation of the AhR complex to the nucleus and interaction with xenobiotic response elements (XRE) in the gene promoter. Subsequent recruitment of coactivators and transcription factors results in transactivation (11). Induction of CYP1A, CYP1B, GSTA, NAD(P)H:quinone oxidoreductase (NQO1) and UGT is mediated through the AhR (12). The inducing potency of indoles is correlated to their AhR affinity (13).

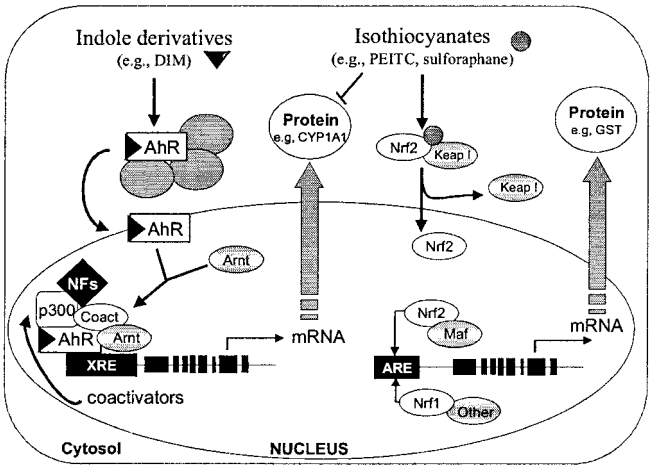

FIGURE 1 Regulation of expression of biotransformation enzymes by indole derivatives and isothiocyanate (ITC) from cruciferous vegetables (adapted from Refs. 11,12,50). Abbreviations: Arnt, AhR nuclear translocator; CYP1A1, cytochrome P450 1A1; Nrf2, NF-E2 related factor; Keap I, Kelch-like ECH-associated protein 1, where ECH is chicken Nrf2.

In contrast, ITC typically activate genes via the antioxidant/electrophile response element (ARE/EpRE) (13,14). PEITC and sulforaphane dissociate the cytoplasmic-anchoring protein Kelch-like ECH-associated protein 1 (Keap I) from the transcription factor Nrf2, allowing it to translocate to the nucleus and to form Nrf2/Maf heterodimers, which activate transcription through ARE/EpRE (14). Regulation of NQO1, γ-glutamylcysteine synthase and several GST is mediated through the ARE/EpRE (12).

Some ITC induce phase I enzymes, others induce only phase II enzymes, and some induce both (4,15,16). Generally, compounds that induce both phase I (e.g., XRE-driven) and phase II (e.g., ARE-driven) steps are thought to speed carcinogenic compounds through the metabolic pathway toward elimination, whereas agents that induce XRE-driven gene expression without stimulating ARE-driven expression are thought to accelerate, rather than retard, chemical carcinogenesis (13). However, the situation is substantially more complex, because not all AhR ligands promote neoplastic disease and promoter regions of some human biotransformation enzymes (e.g., *NQO1*) contain both a XRE and an ARE (13). In addition, in animal models and cell systems combinations of ITC confer protection against genotoxic agents at levels that individual compounds do not achieve alone (13,17). Because a particular *Brassica* species can contain a dozen different glucosinolates (5), a diet high in a variety of glucosinolate-containing vegetables may also exert synergistic effects toward a lower-risk enzyme profile in humans.

Cruciferous vegetables modulate biotransformation pathways: human intervention studies

Over two decades of research have demonstrated that, in humans, commonly consumed cruciferous vegetables and their isolated constituents (e.g., I3C) can affect the CYP1A family and the two major phase II enzyme systems (e.g., GST and UGT) (reviewed in Ref. 18) and alter steroid hormone metabolism (19–21). Human intervention studies have also examined directly effects of cruciferous vegetable supplementation on metabolism of carcinogens. Addition of watercress to diets of smokers significantly increased glucuronidation of nicotine and tobacco-carcinogen metabolites although had little effect on oxidative metabolism (22,23). Similarly, broccoli and Brussels sprouts increased metabolism of cooked meat-derived heterocyclic aromatic amines (i.e., reduced urinary excretion of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and 2-amino-1-methyl-6-phenylimidazol[4,5-*b*]pyridine), implicating the induction of both CYP1A2 and phase II enzymes involved in heterocyclic amine metabolism (16).

Genetic polymorphisms and response to cruciferous vegetable intake

Epidemiologic studies. In general, there is an inverse association between cruciferous vegetables and risk of cancer (reviewed in Refs. 4 and 24). Nonetheless, emerging data from molecular epidemiologic studies suggest that genetic and associated functional variations in biotransformation enzymes lead to individual differences in cancer risk in response to cruciferous vegetable exposure. This relationship has been most extensively studied in relation to *GST*; however, genetic polymorphisms that affect expression of transcription factors or ligand-binding affinity of receptors may also alter the chemopreventive effects of crucifers (Fig. 2).

Null genotypes for *GSTM1* and *GSTT1* result in absence of the respective enzymes. Both of these enzymes are involved in metabolism of environmental carcinogens and reactive oxygen species. Thus, until recently the primary hypothesis has been that individuals with the *GST*-null genotypes are at higher risk for cancer because of reduced capacity to dispose of activated carcinogens. Numerous epidemiologic studies have focused on interactions between these polymorphisms and carcinogen exposure (25,26). Now researchers are also studying relationships between *GST* polymorphisms and exposure to preventive agents (i.e., ITC), with the hypothesis being that, because ITC are metabolized by GST, polymorphisms associated with reduced GST activity will result in longer circulating half-lives of ITC and potentially greater chemoprotective effects of cruciferous vegetables.

Several case-control studies provide evidence that *GST* polymorphisms in conjunction with cruciferous vegetable intake are important risk factors for cancer or precancerous lesions. In 1998 Lin et al. (27) reported that individuals with the highest quartile of broccoli intake had the lowest risk for colorectal adenomas compared with individuals who reportedly never ate broccoli; this inverse association was observed only in those with the *GSTM1*-null genotype. Similarly, colon cancer risk was altered by cruciferous vegetable intake in particular subgroups defined by age, smoking status and *GSTM1* genotype (15). In one study of lung cancer, this relationship was observed among current, but not former, smokers; ITC intake, in combination with the *GSTM1*-null genotype, was protective (28). However, among never-smokers, higher ITC intake was also associated with reduced risk of lung cancer in *GSTM1*- and/or *GSTT1*-null individuals (29,30), suggesting that protective effects of ITC are not limited to their capacity to alter metabolism of tobacco-related carcinogens.

Using urinary biomarkers of cruciferous vegetable exposure has further strengthened the understanding of this gene-diet interaction. London et al. (31) reported that detectable urinary dithiocarbamate (ITC-metabolite) levels were inversely associated with lung cancer risk in men with the homozygous deletion of *GSTM1* or *GSTT1*. Another study indicated that urinary excretion of ITC was higher among *GSTT1*-positive, relative to *GSTT1*-null, individuals, but that *GSTM1* and *P1* genotypes had no effect in this population (32). These data support the in vitro evidence that both GSTM1 and T1 metabolize ITC and that the combination of cruciferous vegetables and the *GST* genotypes may modify cancer risk; nonetheless, the extent to which each isozyme contributes in vivo to ITC exposure remains unclear.

Polymorphisms in enzymes modulated by ITC also have the potential to influence cancer risk. One example is CYP1A2, which activates various procarcinogens, such as heterocyclic amines, nitrosamines and aflatoxin B_1, as well as some endogenous sex steroid hormones implicated in cancer risk (33,34). Thus, individual differences in CYP1A2 activity may also influence individual cancer susceptibility (35).

Cruciferous vegetable supplementation increases CYP1A2 activity under controlled dietary conditions, but no association has been observed overall between cruciferous vegetable intake and CYP1A2 activity in observational studies (36,37). Given the diametrically opposed effects of, for example, cruciferous and apiaceous (carrot family) vegetables on CYP1A2 activity (38) and the high likelihood of confounding between cruciferous and apiaceous vegetable intake in a free-living population (i.e., broccoli eaters will be carrot eaters), this association is likely difficult to detect in observational studies. Nonetheless, in one study, among frequent consumers of broccoli, *GSTM1*-null individuals had a 21% higher CYP1A2 activity than non-null people (39).

Two polymorphisms in *CYP1A2-CYP1A2*1C*, a guanine-to-adenine point mutation in the 5′-flanking region (40) and *CYP1A2*1F* in intron 1 (41), affect enzyme inducibility. Using caffeine metabolite ratios to measure CYP1A2 activity, Nakajima et al. (40) demonstrated that smoking increased CYP1A2 activity only in the *CYP1A2*1C* G/G genotype (homozygous wild type). For *CYP1A2*1F* no genotype differences in CYP1A2 activity were found in nonsmoking individuals (i.e., with uninduced CYP1A2 levels); however, in smokers activity was 1.6-fold higher in the A/A (homozygous wild type) compared with the other genotypes (41). These studies argue for genetically determined differences in response to inducing agents. To date, no studies have examined the effect of these genotypes in connection with cruciferous vegetable intake.

Genetic polymorphisms in factors that regulate gene expression of biotransformation enzymes may also be determinants of cancer risk (42). For example, polymorphisms in the *AhR* gene have been proposed to alter CYP1A1 activity in smokers, although the studies to date have been inconsistent (43,44). Moreover, in mice lacking the Nrf2 transcription factor gene the anticarcinogenic efficacy of the chemopreventive agent oltipraz is lost due to impaired induction of GST and NQO1 (42) and, similarly, the phase II enzyme inducing effect of 6-methylsulfinylhexyl ITC is abrogated (45). Thus, in humans, polymorphisms that affect expression of receptors and transcription factors may impart differential protection by ITC; these remain to be investigated.

Experimental studies

Few human dietary interventions designed to test the effects of diet on biotransformation enzymes have examined the effects of genetic polymorphisms on response to diet (46–48), and to date only one study has tested gene-crucifer interactions. This controlled feeding study tested a priori if *GSTM1* genotype affects response to a diet high in cruciferous vegetables (49). Men and women, recruited on the basis of their *GSTM1* genotype, completed a randomized crossover study of four controlled diet treatments comprised of a basal diet with no vegetables or fruit and the basal diet supplemented with *a*) cruciferous, *b*) allium or *c*) apiaceous vegetables. Serum GSTα concentration, a surrogate measure of hepatic GSTα and an enzyme induced by ITC, increased significantly in response to cruciferous vegetable feeding, but only in *GSTM1*-null individuals. Conversely, among *GSTM1*$^+$ individuals GSTμ activity in leukocytes increased in response to both cruciferous and allium vegetable supplementation. Despite the observational evidence for an effect of *GSTM1* on CYP1A2 response to broccoli (39), the increased CYP1A2 activity on the crucifer-containing diet was not affected by *GSTM1* genotype (38). In conclusion, relationships between cruciferous vegetable intake and cancer risk are influenced by genetic polymorphisms in biotransformation enzymes that metabolize ITC (e.g., GST), as well as possibly in receptors and transcription factors that interact with these compounds.

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