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Modulation of Biotransformation Enzymes by Phytochemicals: Impact of Genotypes

Johanna W. Lampe

Interdisciplinary Graduate Program in Nutritional Sciences, Department of Epidemiology, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA
E-mail: jlampe@fhcrc.org

ABSTRACT

Modulation of biotransformation enzymes is one mechanism by which a diet high in fruits and vegetables may influence cancer risk. Inhibition of cytochrome P450s (CYP) and concomitant induction of conjugating enzymes are hypothesized to reduce the impact of carcinogens in humans. Thus, exposure to types and amounts of phytochemicals may influence disease risk. Like other xenobiotics, many classes of phytochemicals are rapidly conjugated with glutathione, glucuronide, and sulfate moieties and excreted in urine and bile. In humans, circulating phytochemical levels vary widely among individuals even in response to controlled dietary interventions. Polymorphisms in biotransformation enzymes, such as the glutathione *S*-transferases (GST), UDP-glucuronosyltransferases (UGT), and sulfotransferases (SULT), may contribute to the variability in phytochemical clearance and efficacy; polymorphic enzymes with lower enzyme activity prolong the half-lives of phytochemicals *in vivo*. Isothiocyanates (ITC) in cruciferous vegetables are catalyzed by the four major human GSTs; however reaction velocities of the enzymes differ greatly. In some observational studies of cancer, polymorphisms in the *GSTM1* and *GSTT1* genes that result in complete lack of GSTM1-1 and GSTT1-1 protein, respectively, confer greater protection from cruciferous vegetables in individuals with these genotypes. Similarly, we have shown in a controlled dietary trial that levels of GST-alpha – induced by ITC – are higher in GSTM1-null individuals exposed to cruciferous vegetables. The selectivity of glucuronosyl conjugation of flavonoids is dependent both on flavonoid structure as well as on the UGT isozyme involved in its conjugation. The effects of UGT polymorphisms on flavonoid clearance have not been examined; but polymorphisms affect glucuronidation of several drugs. Given the strong interest in the chemopreventive effects of flavonoids, systematic evaluation of these polymorphic UGTs and flavonoid pharmacokinetics are warranted. Overall, these studies suggest that for phytochemicals that are metabolized by, and affect activity of, biotransformation enzymes, interactions between genetic polymorphisms in the enzymes and intake of the compounds should be considered in studies of cancer risk. Genetic polymorphisms in biotransformation enzymes may account in part for individual variation in metabolism of a wide range of phytochemicals and their ultimate impact on health.

INTRODUCTION

Thousands of biologically active phytochemicals have been identified in plant foods, e.g., grains, nuts, legumes, vegetables, and fruit, and are being incorporated routinely into functional foods and dietary supplements. For many of these, researchers are just beginning to understand the factors that influence availability, metabolism, and ultimately exposure in humans. Studies often show wide ranges in biologic responses across individuals when phytochemicals are administered under controlled dietary conditions. This suggests that genetic differences in

handling of, or response to, these bioactive compounds may influence their action. However, very few studies have evaluated the effects of interactions of genotypic differences and phytochemical exposure.

Detoxification or biotransformation enzymes are essential for the metabolism of many important endogenous compounds and in the detoxification of numerous xenobiotics (1). Phase I enzymes such as cytochrome P450-dependent monooxygenases, which catalyze oxidation, hydroxylation, and reduction reactions, convert hydrophobic compounds to reactive electrophiles in preparation for reaction with water-soluble moieties to improve excretion. Phase II enzymes, such as glutathione *S*-transferases (GST), UDP-glucuronyltransferases (UGT), and sulfotransferases (SULT), catalyze these conjugation reactions. The capacity to conjugate metabolically activated intermediates and excrete them from the body is critical in protecting against many potential mutagens. Consequently, research efforts have focused on determining how vegetable and fruit constituents can influence the phase II conjugating enzymes. Nonetheless, it is likely the combined balance of modulation of phase I and II enzymes that affords the greatest protection.

Polymorphisms in genes encoding for proteins that metabolize and transport dietary chemopreventive agents, as well as carcinogens, can affect risk. For example, many classes of phytochemicals are conjugated rapidly with glutathione, glucuronide, or sulfate moieties and excreted in urine and bile. Polymorphisms in GST, UGT, and SULT, may contribute to variability in phytochemical clearance and efficacy; polymorphic enzymes with lower enzyme activity prolong the half-lives of phytochemicals *in vivo* (Fig. 1). Furthermore, polymorphisms that influence receptors and transcription factors that interact with these compounds may affect the impact of the agents on transcriptional regulation.

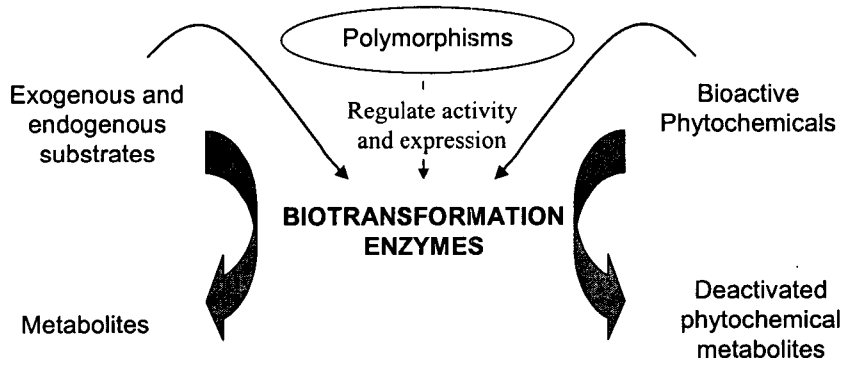

Fig. 1. Relationships between constituents of plant foods, associated metabolites, and polymorphic biotransformation enzymes.

Cytochrome P-450s

Numerous constituents of plant foods, including flavonoids (2), isothiocyanates (3), and allyl sulfides (4), have been found to be potent modulators of the cytochrome P450 monooxygenases (CYP) *in vitro* and in animal models. However, the effects of some of these phytochemicals on CYP are complex. They have the capacity to inhibit certain enzymes at high concentrations of the compound, and to activate moderately the same enzyme at lower concentrations (5). Furthermore, others may be competitive inhibitors of P450s; even when present at low concentrations and in combination with other compounds, their actions can be significant (6).

Numerous dietary interventions have evaluated the effects of plant-food constituents on CYP activities [reviewed in (7)]; however, none have examined the interactions of polymorphic CYP and response to phytochemical

exposure and only a few small-scale epidemiologic studies have tested these interactions (8,9). This is a research area that warrants more attention.

Glutathione *S*-transferases

The glutathione *S*-transferases (GST) are probably among the most studied genes related to functional polymorphisms and cancer risk. These multigene families of enzymes are involved in the conjugation of electrophiles of both exogenous and endogenous origin. Null genotypes for *GSTM1* and *GSTT1* result in lack of the respective enzymes. Both of these enzymes are involved in metabolism of a wide range of compounds, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species. Several phytochemicals, such as the isothiocyanates in cruciferous vegetables, are substrates for *GSTM1* and *GSTT1* (10); conjugation with glutathione and further metabolism yields water-soluble compounds that are excreted in urine. Thus, among the GSTM1-null and GSTT1-null individuals, isothiocyanates may be metabolized more slowly and have the potential to impart a greater chemoprotective effect.

Several case-control studies provide evidence that *GST* polymorphisms in conjunction with cruciferous vegetable intake are important risk factors for some cancers or precancerous lesions [reviewed in (11)]. Using urinary biomarkers of cruciferous vegetable exposure further strengthened the understanding of this gene-diet interaction. London et al. (12) reported that detec

table urinary dithiocarbamate (isothiocyanate-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 not effect in this population (13).

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 (14,15). Nonetheless, in one study, among frequent consumers of broccoli, *GSTM1*-null individuals had a 21% higher CYP1A2 activity than non-null (16). This study is a example of the complex interactions of dietary exposures and multiple polymorphic biotransformation enzymes.

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. A controlled feeding study tested *a priori* if *GSTM1* genotype affects response to a diet high in cruciferous vegetables (17). Men and women, recruited on the basis of their *GSTM1* genotype, completed 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 GST concentration, a surrogate measure of hepatic GST α 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.

UDP-glucuronosyltransferases

UDP-glucuronosyltransferases (UGT), in concert with other biotransformation enzymes such as the cytochrome P450 enzymes, play an important role in detoxification and chemoprotection. They catalyze the transfer of the glucuronyl group from uridine 5'-disphosphoglucuronic acid to endogenous molecules (e.g., bilirubin and steroid hormones) and exogenous substrates (e.g., drugs, phytochemicals, environmental pollutants, and carcinogens). The

resulting glucuronide products are more polar, generally water-soluble, less toxic, and more easily excreted than the substrate molecules. In humans, two UGT families have been classified by sequence identities [for review see (18)]. Generally, family 1 enzymes catalyze the glucuronidation of bilirubin and xenobiotic phenols, as well as some steroids, while family 2 conjugates primarily steroids with glucuronide (19). The human *UGT1* gene is located on chromosome 2 and is unique, consisting of at least 12 different promoters and first exons which can be spliced to the common exons 2 through 5 (20). This results in the UGT1 isozymes sharing the C-terminal 246 amino acid residues, but having variable N-termini of approximately 285 amino acids. Substrate-binding specificity is provided by the N-terminal half of the protein and UDP-glucuronic acid binding occurs in the C-terminal portion (20).

Several genetic variants in the *UGT1* and *UGT2* families have been identified that alter UGT activity to varying degrees in humans (21-23); however, few studies have examined UGT genotype-diet interactions. A polymorphism in the promoter sequence upstream of *UGT1A1 (UGT1A1*28)* is the genetic basis for benign unconjugated hyperbilirubinemia associated with reduced hepatic UGT conjugation of bilirubin (Gilbert's syndrome) (24). Increased numbers of thymine-adenine (TA) repeats are correlated inversely with *UGT1A1* promoter activity and directly with serum total bilirubin concentrations (24). Although UGT1A1 is the major isoform responsible for glucuronidation of bilirubin in human liver, it is capable of conjugating various phenols, anthraquinones, and flavones, many of which are found in foods, e.g., emodin (rhubarb), naringenin (grapefruit), eugenol (cloves) (25). Thus, polymorphisms in *UGT1A1* may influence bilirubin clearance and exposure to xenobiotics.

Recently, we investigated, in an observational study, whether foods from four botanical groups, (*Cruciferae* (e.g., broccoli), *Rutaceae* (citrus), *Liliaceae* (e.g., onions), and *Leguminosae* (legumes), were associated with increased UGT1A1 activity as indicated by serum bilirubin concentrations and whether the effect varied by *UGT1A1*28* genotype, comparing those homozygous for the $[TA]_7$-repeat allele (7/7) to homozygous wild-types (6/6) and heterozygotes (6/7) combined. There was a significant inverse association between total, direct and indirect bilirubin and interaction of *UGT1A1*28* genotype with *Cruciferae* intake. Individuals with the 7/7 genotype had reduced bilirubin concentrations with increased intake of cruciferous vegetables, whereas individuals with the 6/6 or 6/7 genotype did not. With regard to UGT1A1-conjugated carcinogens (e.g., heterocyclic amines, polycyclic aromatic hydrocarbons), individuals with decreased UGT1A1 activity due to the 7/7 genotype may be at greater risk for carcinogenesis, but our results imply that they also may have greater opportunity to decrease that risk through dietary intervention.

Among the UGT2B family, *UGT2B15* is expressed in liver, kidney, testis, mammary gland, prostate, and lung (23). This isozyme catalyzes the glucuronidation of a wide range of substrates, including simple phenolic compounds, coumarins, flavonoids, anthraquinones, drugs and C_{19} steroids, such as testosterone and dihydrotestosterone (DHT) (26,27). Nucleotide differences in the coding region of *UGT2B15* lead to two forms of the enzyme that have similar substrate specificities and K_m values; however, UGT2B15(Y^{85}) has a higher V_{max} than UGTB15(D^{85}) for 3-α-diol and DHT (23). This polymorphism may contibute to individual variability in xenobiotic glucuronidation. Decreased conjugation of specific phytochemicals by a polymorphic UGT2B15 may result in altered activity of other UGT isoforms. Thus, understanding the relationships between *UGT* polymorphisms and phenotypes is going to be a key component in establishing what *UGT* gene-diet interactions contribute to disease risk and to variations in response to drug or diet therapy.

Sulfotransferases

Sulfotransferases (SULT) catalyze sulfate conjugation, an important pathway in the biotransformation of neurotransmitters, hormones, drugs, and xenobiotics. Conjugation with a charged sulfonate moiety usually decreases the biologic activity of the compound and increases its aqueous solubility and excretion. Several dietary flavonoids, including the soy isoflavones genistein and daidzein, and the tea flavonoid (-)-epicatechin are sulfated *in vivo. In vitro*, many of these flavonoids have been shown to be potent mixed inhibitors of hepatic estrogen sulfotransferase at low concentrations, and to have modest effects on monoamine phenolsulfotransferase at higher concentrations (28). Thus, these compounds have the potential to influence bioavailability of endogenous estrogens by altering ratios of active and inactive steroid hormones in human tissues, but this remains to been evaluated.

CONCLUSION

Overall, these studies suggest that for phytochemicals that are metabolized by, and affect activity of, biotransformation enzymes, interactions between genetic polymorphisms in the enzymes and intake of the compounds should be considered in studies of disease risk. Genetic polymorphisms in biotransformation enzymes may account in part for individual variation in metabolism of a wide range of phytochemicals and their ultimate impact on health. Controlled dietary interventions provide a useful approach to testing genotype-phytochemical interactions.

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