

Editorial

Dietary Isothiocyanates as Confounding Factors in the Molecular Epidemiology of Colon Cancer

Commentary re: H. J. Lin *et al.*, Glutathione Transferase Null Genotype, Broccoli, and Lower Prevalence of Colorectal Adenomas. *Cancer Epidemiol. Biomark. Prev.*, 7: 647–652, 1998

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In this issue, Lin *et al.* (1) show convincing statistics that subjects exposed to dietary broccoli who have a GSTM1² null phenotype are less susceptible to colon cancer than those who are GSTM1 positive. The expected effect of a GSTM1 null phenotype is greater, not less, susceptibility to cancers due to reduced carcinogen detoxification by GSTM1 (2, 3).

Broccoli reverses this outcome for two reasons: (a) it contains sulforaphane, a cancer chemopreventive isothiocyanate (4) that present evidence indicates both inhibits cytochrome P450 (and therefore carcinogen activation) and induces GSTs, consequently increasing carcinogen detoxification (5–7); and (b) sulforaphane is itself a substrate for GSTs, which may abolish its anticarcinogenic activity (8, 9). Because Lin *et al.* suggest that their findings are the consequence of reduced conjugation of sulforaphane among GSTM1 null subjects (1), this commentary will attempt to assemble data available relating to this hypothesis to assess its validity.

Their study has an interesting practical outcome because dietary isothiocyanates have rarely, if ever, been taken into account in investigations of the molecular epidemiology of cancer, and it is probable that they have been confounding factors in a number of studies (3) and should be borne in mind in the design of future protocols.

Lin *et al.* (1) have observed a significant effect of a broccoli diet on susceptibility to colon cancer, even without taking into consideration exposure to specific colon carcinogens. To analyze the processes upon which their findings might be based, it is necessary to select a known colon carcinogen with a reasonably well-understood pharmacology, such as the food-borne heterocyclic amine PhIP, which has been associated with human colon carcinogenesis (10).

GSTA1, GSTA2, and GSTM1 (in those individuals that have the gene) are most abundant in the liver and small intestine but low in occurrence in colon (11). PhIP in food enters the circulation via the small intestine and passes immediately to the liver. The liver, taking into account its large mass and rich

content of enzymes of xenobiotic metabolism, has greater power, by orders of magnitude, to both activate and detoxify carcinogens, compared with other tissues.

PhIP is activated by hepatic CYP1A2 catalyzed *N*-hydroxylation, followed by *O*-acetylation, and is detoxified by reduction to the parent amine by GSTA1 (10). *N*-Acetoxy-PhIP may also form unstable adducts with GSH and other thiols and be degraded to 5-hydroxy-PhIP (9). It is envisioned that a proportion of *N*-acetoxy-PhIP escapes detoxification and enters the circulation to cause cancer in extrahepatic tissues, which have a low or negligible content of GSTs (12) that might detoxify it, such as the colon.

We know that *N*-acetoxy-PhIP has a degree of stability in the blood, but this has been little studied (13); it is probably reasonable to use BPDE, one of the most studied of all aromatic carcinogens, as an analogue. For example, once in the circulation BPDE is protected by binding to serum albumin and lipoproteins such that it has a half-life of ~30 s (14).

Because the circulation time of the blood has been estimated at 10–30 s, according to whether the subject is exercising or at rest (15), BPDE and probably *N*-acetoxy-PhIP forming in the liver should reach all parts of the body in the first pass at least. *In vivo* extrahepatic transport of BPDE and tissue uptake from the circulation have been elegantly demonstrated in the mouse. After BPDE was added to serum and administered *i.v.*, serum BPDE levels were shown to fall rapidly and to be associated with a simultaneous increase in cellular BPDE-DNA adducts. As predicted above, BPDE-DNA adducts were found in tissues that do not themselves activate benzo(*a*)pyrene (16). Similar conclusions were drawn from experiments with human cells (17).

Having established a possible means for the transport of *N*-acetoxy-PhIP from the liver, we are now in a position to consider the influence of the broccoli diet and its constituent sulforaphane upon the amounts of *N*-acetoxy-PhIP leaving the liver. GSTM1 uses sulforaphane as a substrate and, when present, reduces levels of sulforaphane available for the inhibition of the activation of *N*-acetoxy-PhIP and the induction of its detoxification. The following facts concerning GSTM1-dependent metabolism of isothiocyanates are important for the understanding of the results of the report by Lin *et al.* (1). The metabolic reaction is reversible, and the rate and K_m of both conjugate formation and its reversion are greater with GSTM1 than with GSTA1. As a result, GSTM1 converts sulforaphane to the GSH conjugate more rapidly than does GSTA1. Consequently, GSTM1 makes more sulforaphane available for excretion via the GSH pump. In contrast, GSTA1 sequesters the conjugate and catalyzes its reversion to sulforaphane at a slow rate. Sufficient kinetic data for the GSH conjugation of sulforaphane and other chemopreventive analogues such benzyl and

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² The abbreviations used are: GSTM1, GSTA1, and GSTA2, glutathione S-transferase M1, A1, and A2, respectively; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine; GSH, reduced glutathione; BPDE, benzo(*a*)pyrene-7,8-diol-9,10-oxide.

phenethyl isothiocyanates are not available to make a critical kinetic analysis possible (8, 9). However, the present view is that in the GSTM1-positive subject, the conjugation and excretion of sulforaphane is favored, with resulting diminution of chemoprevention. In the GSTM1-null individual, sulforaphane tends to be conserved as GSTA1-sequestered conjugate and converted slowly to the active isothiocyanate. Using benzyl isothiocyanate and GSTA1 as a model system, it has been calculated that at equilibrium ~1% is present as the unconjugated isothiocyanate (9). It is apparent that the targets responsible for control of expression of the enzymes concerned are very reactive and are affected at low doses of isothiocyanate. As mentioned above, studies with human hepatocytes in primary culture show that the major effect is on gene expression (4) rather than directly upon the enzymes.

The findings of reduced risk of colon adenomas among high consumers of broccoli who are GSTM1 null by Lin *et al.* (1) may explain some of the inconsistencies in studies of the effect of GSTM1 genotypes on susceptibility to cancer (3). The revelation of an unexpected confounding factor in the diet serves to illustrate the extremely complex mechanism and multiple factors involved in carcinogenesis and the important challenges in the field of molecular epidemiology.

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