Short Communication

Absence of the Glutathione S-Transferase M1 Gene Increases Cytochrome P4501A2 Activity among Frequent Consumers of Cruciferous Vegetables in a Caucasian Population

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Abstract

The cancer protective effect of cruciferous vegetables has been attributed to induction of phase II enzymes. But cruciferous vegetables also induce cytochrome P4501A2 (CYP1A2), which catalyzes the metabolic activation of various procarcinogens, including aromatic amines in tobacco. Thus, frequent intake of cruciferous vegetables could also result in cancer-enhancing effects. GSTM1 is involved in the detoxification of various carcinogens, but it also enhances the excretion of isothiocyanates and possibly other enzyme inducers in cruciferous vegetables. We, therefore, hypothesized that GSTM1 null genotype might be associated with increased CYP1A2 activity among frequent consumers of cruciferous vegetables because the excretion of CYP1A2 inducers contained in these vegetables may be partially inhibited in the absence of the GSTM1 enzyme.

Three hundred twenty-eight non-Hispanic white residents of Los Angeles County (265 males and 63 females) were genotyped for the presence or absence of GSTM1 alleles and phenotyped for CYP1A2 activity. Information on usual dietary habits was obtained from these subjects through in-person interviews. Among frequent (at least once a week) consumers of broccoli, GSTM1 null individuals exhibited a 21% higher geometric mean level of CYP1A2 activity relative to GSTM1 non-null individuals (5.24 versus 4.32, two-sided P = 0.01). No such difference was observed in subjects who consumed broccoli less frequently (two-sided P = 0.39). This interactive effect of GSTM1 genotype and vegetable intake on CYP1A2 activity also was observed when overall intake of the five cruciferous vegetables under study (broccoli, cabbage, cauliflower, Brussels sprouts, and mustard greens) was examined. Among weekly consumers of cruciferous vegetables, GSTM1 null individuals showed a 16% higher geometric mean level of CYP1A2 activity relative to GSTM1 non-null individuals (5.03 versus 4.33, two-sided P = 0.02), whereas no difference was evident among those who consumed cruciferous vegetables less frequently (two-sided P = 0.35). Our results suggest that cruciferous vegetables contain CYP1A2 inducers, which are deactivated in the presence of GSTM1.

Introduction

The often observed cancer protective effect of cruciferous vegetables has been attributed mainly to their induction of various phase II enzymes involved in the detoxification of carcinogens (1). However, several metabolic experiments conducted in human subjects have consistently observed increased CYP1A2 activity after intake of cruciferous vegetables (2). Because CYP1A2 catalyzes the metabolic activation of various procarcinogens (3), these latter data suggest that cruciferous vegetable intake may also be associated with cancer-enhancing effects.

GSTM1 is a member of the GST family of phase II detoxifying enzymes that promote the conjugation of a wide range of reactive metabolites to glutathione (4). GSTM1 is also involved in the excretion of isothiocyanates, a group of enzyme inducers in cruciferous vegetables (4). GSTM1 is polymorphic in humans, with inherited homozygous deficiency (−/−) being associated with no GSTM1 enzymatic activity in affected individuals (4). We hypothesized that among frequent consumers of cruciferous vegetables, those possessing the GSTM1 null genotype may exhibit higher CYP1A2 activity relative to those who are GSTM1-positive because the latter group of individuals may have higher excretion rates of CYP1A2 inducers that are present in cruciferous vegetables. We would expect no difference in CYP1A2 activity among infrequent consumers of cruciferous vegetables. We explored this hypothesis in 328 non-Hispanic white residents of Los Angeles County who were between the ages of 30 and 72 years.

Materials and Methods

Subjects. The subjects included in this analysis were the first 328 non-Hispanic white control subjects recruited for an ongoing case-control study of bladder cancer in Los Angeles County. These control subjects were selected from the nei-

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3 The abbreviations used are: CYP1A2, cytochrome P4501A2; GSTM1, glutathione S-transferase M1; AAMU, S-acetylamino-6-amino-3-methyluracil; IX, 1-methylxanthin; 1U, 1-methylurate; 17U, 8-hydroxyxparaxanthin.
Laboratory Tests. Urine samples identified only by code summed each of the cruciferous vegetable items 2 years prior to in-person interviews. Each subject was asked to indicate how to 6.5 among the subjects, whereas urine volume ranged from CYP1A2 phenotype determination. Urine pH ranged from 4.4 urine) within 24 h of collection and stored at -20°C until thine and urate assays; thus, the presence of this compound in consumption (>300 mg or >4 cups of coffee) has been shown to acetaminophen on the previous day. Excessive caffeine con-

The subject collected an overnight urine sample (ending with coffee prepared from two packets of instant coffee (about 70 collected from each study subject. Subjects were asked to

the past 60 days, a blood and an overnight urine specimen were

the prescribed packets of instant coffee) and use of

The subject was asked about tobacco use in neighborhoods where bladder cancer cases resided at the time of cancer diagnosis and were individually matched to the index cases by age (within 5 years), sex, and race. Eighty-one % (n = 265) of the subjects were men. 44% completed college or graduate school, and 90% were born in the United States.

Prior to participation, all subjects signed an informed consent form approved by the University of Southern California Institutional Review Board. In addition to an in-person interview during which the subject was asked about tobacco use in the past 60 days, a blood and an overnight urine specimen were collected from each study subject. Subjects were asked to refrain from chocolate and to limit their caffeine intake to no more than four cups of coffee (or 10 cans of soda) on the day prior to the overnight urine collection and to drink a cup of coffee prepared from two packets of instant coffee (about 70 mg of caffeine) given by us between 3:00 p.m. and 6:00 p.m. The subject collected an overnight urine sample (ending with the first morning void) that was picked up and immediately processed. At the time of collection of the urine specimen, the subject was briefly interviewed about caffeine intake (in addition to the prescribed packets of instant coffee) and use of acetaminophen on the previous day. Excessive caffeine consumption (>300 mg or >4 cups of coffee) has been shown to affect the validity of the CYP1A2 genotyping assay. Acetaminophen is used as the internal standard for the methylxanthine and urate assays; thus, the presence of this compound in urine may affect the validity of these assays (5). The urine specimens were acidified (20 mg of ascorbic acid per ml of urine) within 24 h of collection and stored at -20°C until CYP1A2 phenotype determination. Urine pH ranged from 4.4 to 6.5 among the subjects, whereas urine volume ranged from 130 to 2260 ml.

Information about the usual adulthood intake of broccoli, cabbage/cole slaw (cabbage), cauliflower, Brussels sprouts, and collard greens/mustard greens/turnip greens/kale (mustard greens), which are the commonest cruciferous vegetables in the United States diet, was collected from study subjects during the in-person interviews. Each subject was asked to indicate how many times per day, week, month or year he/she usually consumed each of the cruciferous vegetable items 2 years prior to the year of cancer diagnosis of the index case.

Laboratory Tests. Urine samples identified only by code numbers were sent frozen to the Ohio State University Laboratory. The samples were kept at -80°C until analysis according to the method of Kalow and Tang (5). Higher values of the CYP1A2 index reflect higher CYP1A2 activities. DNA was extracted from whole blood and GSTM1 genotyping (null versus non-null) was performed according to the method as described by Bell et al. (6).

Statistical Analysis. The distribution of the CYP1A2 index, i.e., ratio of (AAMU + 1X + 1U)/17U (the CYP1A2 index) in study sub-
eluded in all analyses presented in this report. The CYP1A2 index measured in our study population ranged from 1.41 to 25.90; the geometric mean CYP1A2 index was 4.73 (geometric SD, 1.65).

Fifty-eight subjects were current tobacco smokers. The tobacco contents of one cigar and one pipe were assumed to be equivalent to the content of 4.5 and 2.5 cigarettes, respectively, for the four cigar and three pipe smokers. There was a statistically significant association between level of CYP1A2 index and intensity of smoking (P < 0.0001). The geometric mean CYP1A2 indices (and 95% confidence intervals) among non-smokers (n = 270), moderate smokers (n = 28) (<20 cigarette equivalents/day), and heavy smokers (n = 30) were 4.30 (4.07−4.54), 6.58 (5.57−7.78), and 8.29 (7.05−9.74), respectively. Neither age nor gender had a statistically significant influence

| Table 1 Smoking-adjusteda geometric mean levels of (AAMU + 1X + 1U)/17U (the CYP1A2 index) in study subjects by intake frequencies of selected cruciferous vegetables and GSTM1 genotype |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| GSTM1 null | GSTM1 non-null | GSTM1 null vs. non-null |
| Weekly consumers | Less-frequent consumers | GSTM1 null | GSTM1 non-null | GSTM1 null vs. non-null |
| Broccoli | 5.24 (84)b | 4.32 (86) | 0.01 | 4.57 (85) | 4.87 (73) | 0.39 |
| Cabbage or cole slaw | 4.65 (61) | 4.33 (53) | 0.41 | 5.04 (108) | 4.69 (106) | 0.27 |
| Cauliflower | 5.05 (39) | 4.34 (49) | 0.13 | 4.85 (130) | 4.67 (110) | 0.54 |
| Brussels sprouts | 4.95 (16) | 5.10 (15) | 0.85 | 4.89 (153) | 4.51 (144) | 0.14 |
| Mustard greens | 5.56 (9) | 7.26 (6) | 0.28 | 4.86 (160) | 4.48 (153) | 0.13 |
| Any of the above | 5.03 (108) | 4.33 (103) | 0.02b | 4.66 (61) | 5.05 (56) | 0.35 |

a Adjusted for daily consumption of cigarette equivalents as a continuous variable. The tobacco contents of one cigar and one pipe were assumed to be equivalent to the tobacco contents of 4.5 and 2.5 cigarettes, respectively.

b Number of subjects.

The effect of GSTM1 genotype on CYP1A2 index level differed significantly between subjects who consumed broccoli 1+/week and those who did not. Two-sided P (interaction effect) = 0.01.

The effect of GSTM1 genotype on CYP1A2 index level differed between subjects who consumed any cruciferous vegetables 1+/week versus those who did not. Two-sided P (interaction effect) = 0.037.

Results

Eleven subjects took acetaminophen-containing medication and 7 subjects had excessive caffeine consumption on the day prior to their overnight urine collection. Excluding these 18 subjects did not materially alter our findings; therefore, they were included in all analyses presented in this report. The CYP1A2 index measured in our study population ranged from 1.41 to 25.90; the geometric mean CYP1A2 index was 4.73 (geometric SD, 1.65).

Fifty-eight subjects were current tobacco smokers. The tobacco contents of one cigar and one pipe were assumed to be equivalent to the content of 4.5 and 2.5 cigarettes, respectively, for the four cigar and three pipe smokers. There was a statistically significant association between level of CYP1A2 index and intensity of smoking (P < 0.0001). The geometric mean CYP1A2 indices (and 95% confidence intervals) among non-smokers (n = 270), moderate smokers (n = 28) (<20 cigarette equivalents/day), and heavy smokers (n = 30) were 4.30 (4.07−4.54), 6.58 (5.57−7.78), and 8.29 (7.05−9.74), respectively. Neither age nor gender had a statistically significant influence
Number of subjects. Among weekly eaters of cruciferous vegetables, the effect of this cruciferous vegetable less frequently consumed broccoli at least once a week and those who consumed this cruciferous vegetable less frequently (P for interaction effect = 0.01). Among weekly consumers of broccoli, mean CYP1A2 index in GSTM1 null individuals was 21% higher than that in GSTM1 non-null individuals (5.24 versus 4.32), and the difference was statistically significant (P = 0.01). In contrast, there was no difference in mean CYP1A2 indices between GSTM1 null and GSTM1-positive individuals who consumed broccoli less frequently (P = 0.39). This interaction effect between broccoli intake and GSTM1 genotype on CYP1A2 index level was not present for the other, less frequently consumed cruciferous vegetables. The Ps for interaction were 0.99, 0.33, 0.53, and 0.16 for cabbage, cauliflower, Brussels sprouts, and mustard greens, respectively. When overall intake of cruciferous vegetables was examined, there was again a statistically significant interaction effect (P = 0.03) with GSTM1 genotype on level of CYP1A2 index. Among weekly consumers of any cruciferous vegetables, mean CYP1A2 index in GSTM1 null individuals was 16% higher than that in GSTM1 non-null individuals (5.03 versus 4.33), and the difference was statistically significant (P = 0.02). Among less frequent consumers of cruciferous vegetables, no difference in CYP1A2 activity level was observed between GSTM1 null and GSTM1 non-null individuals (P = 0.35).

Table 2 presents the geometric mean CYP1A2 activity levels among weekly consumers of broccoli (or any cruciferous vegetables) according to GSTM1 genotype for current smokers and nonsmokers separately. The mean levels of CYP1A2 index were about 50–80% higher in smokers than in nonsmokers with comparable GSTM1/vegetable intake profile. Smoking did not modify the association between CYP1A2 index and GSTM1 genotype among weekly consumers of broccoli (P for interaction = 0.37) or among weekly consumers of any cruciferous vegetables (P for interaction = 0.76). In both smokers and nonsmokers, weekly cruciferous vegetable eaters possessing the GSTM1 null genotype exhibited higher mean CYP1A2 activity levels relative to those with similar cruciferous vegetable intake pattern but possessing at least one functional allele of the GSTM1 gene. Thus, smokers having the GSTM1 null genotype had the highest mean CYP1A2 activity level, whereas nonsmokers having at least one functional allele of the GSTM1 gene had the lowest mean level, and the difference was close to 2-fold.

### Discussion

The data are in support of our hypothesis that GSTM1 null individuals exhibit higher CYP1A2 activity than GSTM1-positive individuals consuming comparable amounts of cruciferous vegetables, due to the higher excretion rates of CYP1A2 inducers in cruciferous vegetables among the latter group. The observed GSTM1 genotype effect on CYP1A2 activity was primarily among frequent consumers of broccoli, which was the commonest cruciferous vegetable in this study population. Either the GSTM1 genotype effect on CYP1A2 activity is specific to broccoli, or the finding could simply reflect the higher likelihood of observing an effect in a more highly exposed population.

It is currently unknown what components of cruciferous vegetables (or specifically broccoli) are mainly responsible for CYP1A2 induction. There are the isothiocyanates and indoles, two major degradation products of glucosinolates that are present in cruciferous vegetables. Data from rat and human in vivo studies indicate that indole derivatives can induce CYP1A2 (8, 9), but the role of GSTM1 in their metabolic clearance remains to be established. GSTM1 is known to play an important role in the excretion of isothiocyanates (4). According to rodent experiments, the effect of isothiocyanates on cytochrome P450 enzymes depend on experimental conditions, the specific isothiocyanate involved, the treatment regimen, the target tissue examined, and the specific monooxygenase measured (10). At the present time, there is no clear evidence linking isothiocyanates to CYP1A2 induction in humans. We conjecture that specific isothiocyanates may be mediators of the GSTM1/broccoli effect on CYP1A2 activity. In fact, broccoli has been found to contain 40 and 325% more total isothiocyanates than cabbage and cauliflower, respectively (11).

This is the first population-based study that used a ratio of caffeine metabolites as an index of CYP1A2 activity. Relative to nonsmokers, moderate and heavy tobacco smokers exhibited statistically significant 1.5- and 2-fold increases, respectively, in their mean CYP1A2 indices. Our results agree well with those of previous studies that used the same (5, 12, 13) or different caffeine-based urinary metabolite ratios (3, 14, 15). Smoking is a known inducer of CYP1A2 activity, with polycyclic aromatic hydrocarbons being potential mediators (3). The absence of an age effect on CYP1A2 index and the presence of a small but statistically nonsignificant gender effect also are consistent with previous findings (3, 5, 12, 16).

This novel finding should be replicated in other populations using different phenotyping methods, given the debate over the validity of different CYP1A2 indices (17). The roles of GSTM1 and other members of the GST family in the metabolic clearance of CYP1A2 inducers in cruciferous vegetables...
serve further investigation. Several case-control studies have reported a significant positive association between the GSTM1 null genotype and bladder cancer risk (18). We previously reported an association between GSTM1 null genotype and increased level of aminobiphenyl-hemoglobin adducts (19). Aminobiphenyls, which require bioactivation by CYP1A2 to reach their full carcinogenic potential, are a group of compounds present in cigarette smoke that are believed to be responsible, at least in part, for bladder cancer development in smokers. Thus, our present results raise the possibility that the oft-observed GSTM1 null genotype effect on bladder cancer risk (18) may be mediated, in part, through CYP1A2 induction and increased bioactivation of aminobiphenyls among cruciferous vegetable consumers.

References

Absence of the glutathione S-transferase M1 gene increases cytochrome P4501A2 activity among frequent consumers of cruciferous vegetables in a Caucasian population.

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