Dietary isothiocyanates, glutathione S-transferase polymorphisms and colorectal cancer risk in the Singapore Chinese Health Study

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Dietary intake of cruciferous vegetables (Brassica spp.) has been inversely related to colorectal cancer risk, and this has been attributed to their high content of glucosinolate degradation products such as isothiocyanates (ITCs). These compounds act as anticarcinogens by inducing phase II conjugating enzymes, in particular glutathione S-transferases (GSTs). These enzymes also metabolize ITCs, such that the protective effect of cruciferous vegetables may predicate on GST genotype. The Singapore Chinese Health Study is a prospective investigation among 63 257 middle-aged men and women, who were enrolled between April 1993 and December 1998. In this nested case-control analysis, we compared 213 incident cases of colorectal cancer with 1194 controls. Information on dietary ITC intake from cruciferous vegetables, collected at recruitment via a semi-quantitative food frequency questionnaire, was combined with GSTM1, T1 and P1 genotype from peripheral blood lymphocytes or buccal mucosa. When categorized into high (greater than median) and low (less than/equal to median) intake, dietary ITC was slightly lower in cases than controls but the difference was not significant [odds ratio (OR) 0.81, 95% confidence interval (CI) 0.59–1.12]. There were no overall associations between GSTM1, T1 or P1 genotypes and colorectal cancer risk. However, among individuals with both GSTM1 and T1 null genotypes, we observed a 57% reduction in risk among high versus low consumers of ITC (OR 0.43, 95% CI 0.20–0.96), in particular for colon cancer (OR 0.31, 0.12–0.84). Our results are compatible with the hypothesis that ITCs from cruciferous vegetables modify risk of colorectal cancer in individuals with low GST activity. Further, this gene–diet interaction may be important in studies evaluating the effect of risk-enhancing compounds in the colorectum.

Introduction

The role of diet in the aetiology of colorectal cancer is widely accepted. Among the various components of diet, epidemiologic studies have been fairly consistent in demonstrating an inverse association with intake of cruciferous vegetables (1,2), and this is supported by recent findings from animal studies (3). A distinctive feature of these vegetables is their relatively high content of glucosinolates, which are converted in vivo to isothiocyanates (ITCs), indoles and nitriles by the enzyme myrosinase (4,5). Apart from the colorectum, cruciferous vegetables have been shown to possess chemopreventive activity against a variety of other cancers such as those of the lung and prostate (6,7), and there is accumulating evidence from laboratory studies that this occurs primarily through their effects on the metabolism of pro-carcinogens. Specifically, glucosinolate degradation products from cruciferous vegetables are believed to inhibit phase I activating enzymes, and induce phase II detoxication enzymes (8,9). ITCs, in particular, exert their effects through the latter pathway. Induction of phase II detoxication enzymes reduces exposure of the target tissue to DNA damage, thus exerting a ‘blocking effect’ on the initiation stage of chemical carcinogenesis (5).

One of the most important detoxification enzyme systems is the glutathione S-transferase (GST) family of enzymes. These enzymes are expressed in a wide variety of human tissue, including both normal and malignant colonic mucosa (10). Conjugation with glutathione by GST is an important step in the metabolism and subsequent detoxification of carcinogens like polycyclic aromatic hydrocarbons, among others. They are also known to metabolize ITCs, resulting in the formation of N-acetylase conjugates, which are excreted in the urine (11,12). Human GSTs comprise several subfamilies of isoenzymes: principally GSTM, GSTP and GSTT. Deletions in the GSTM1 and GSTT1 gene produce the null genotypes, which lead to absence of activity of these enzymes; similarly, reduced activity of GSTP1 has been attributed to the low activity B allele (13–15). Epidemiologic studies of GST and colorectal cancer risk have been suggestive of a deleterious effect of the null or low activity genotype (16,17), but findings have been inconsistent (18,19).

Recent findings, both laboratory and epidemiological, suggest that this somewhat equivocal relationship may be explained by a more complex gene–environment interaction. Dietary ITCs induce GST enzyme activity (20,21), and their potency as enzyme inducers has been shown to be related to their total intracellular concentrations (22), and more recently to the formation of intracellular reactive oxygen species (23). On the other hand, the GST enzymes metabolize ITC. The beneficial effect of ITC is therefore dependent in part on the presence or absence of GST activity; individuals with low activity would metabolize these compounds at a slower rate, allowing the protective effects to be exerted to a greater extent at the target tissue level. Similarly, the observed effect of GST on disease risk needs to be viewed in the light of dietary elements known to induce its activity.

The Singapore Chinese population has rising rates of colorectal cancer, particularly cancer of the colon, the age-standardized incidence in males doubling from 10.4 to 20.9 per 100 000 per year in the last three decades (24). Their rates are significantly higher than those in China and similar to Chinese populations in developed countries (25). This population consumes high amounts of cruciferae relative to those in other

Abbreviations: CI, confidence interval; GST, glutathione S-transferase; ITC, isothiocyanates; OR, odds ratio.
developed countries. Within the Singapore Chinese Health Study, we demonstrated previously that at all levels of consumption of cruciferous vegetables; urinary excretion of ITC was dependent on GSTT1 genotype (26).

In this report, we use data from the Singapore Chinese Health Study to examine the association between dietary ITC, and its interaction with GSTM1, T1 and P1 genotypes, and colorectal cancer risk in this population.

Materials and methods

Study population

The subjects were participants of the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk (27).

Briefly, between April 1993 and December 1998, we recruited 63 257 Chinese men and women from two major dialect groups in Singapore (Hokkien and Cantonese). These were between the ages of 45 and 74 years, residing in government housing estates, of which 86% of the population are resident. Each completed a structured questionnaire administered in person by a trained fieldworker.

In April 1994, 1 year after the initiation of the cohort study, we began collection of blood and single-void urine specimens from a random 3% sample of study participants. A 20 ml blood sample was obtained from each subject. Immediately after blood collection, the tubes were put on ice during transport from the subjects’ homes to the laboratory. The specimens were then separated into their various components (plasma, serum, red blood cells and Buffy coat). These were subsequently stored in a liquid nitrogen tank at −180°C until August 2001, when they were moved to −80°C freezers, which were more economical for long-term storage. Subjects who were unwilling to donate blood were asked to donate buccal cells through the use of a mouthwash protocol based on published methods (28,29). These subjects were provided with 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s. The mouthwash was given 10 ml of commercially purchased Listerine mouthwash and asked to vigorously swish the liquid in their mouths for 60 s.
Table I. Dietary ITC intake in relation to risk of colorectal cancer, Singapore Chinese Health Study

<table>
<thead>
<tr>
<th>Dietary ITC intake level ( ^a )</th>
<th>Controls</th>
<th>Colorectal cancer</th>
<th>Colon cancer</th>
<th>Rectal cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI) ( ^b )</td>
<td>OR (95% CI) ( ^b )</td>
<td>OR (95% CI) ( ^b )</td>
<td>OR (95% CI) ( ^b )</td>
</tr>
<tr>
<td><strong>Total subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ITC</td>
<td>599</td>
<td>127</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>High ITC</td>
<td>595</td>
<td>86</td>
<td>0.81 (0.59–1.12)</td>
<td></td>
</tr>
<tr>
<td><strong>Never smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ITC</td>
<td>402</td>
<td>71</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>High ITC</td>
<td>462</td>
<td>57</td>
<td>0.89 (0.58–1.36)</td>
<td></td>
</tr>
<tr>
<td><strong>Ever smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ITC</td>
<td>197</td>
<td>56</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>High ITC</td>
<td>133</td>
<td>29</td>
<td>0.77 (0.44–1.36)</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \)Low (or high) ITC levels were defined as lower (or higher) than the median dietary ITC intake (5.16 \( \mu \)mol/1000 kcal) among all cohort members.

\( ^b \)ORs and 95% CIs were derived from conditional logistic regression models including education, BMI, cigarette smoking, weekly strenuous sports/vigorous work, alcohol drinking, and saturated fat as covariates; each risk set of cases and controls was formed according to sex, dialect group (Cantonese, Hokkien), year of recruitment (1993–1995, 1996–1998), and year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1953).

ever), weekly strenuous sports/vigorous work (yes, no), alcohol drinking (g ethanol/day in four categories) and saturated fat (% kcal in quartiles).

Statistical analysis was carried out using the SAS software version 8.2 (SAS Institute, Cary, NC) and Epilog for Windows version 1.0 (Epicenter Software, Pasadena, CA). All \( P \) values reported are two-sided, and \( P \) values of <0.05 were considered statistically significant.

Results

Altogether, 213 cases, and 1194 controls were included in this analysis. Of the cases, 130 (61%) had cancers of the colon, and the remaining 83 (39%) had rectal carcinomas.

The mean age of cases at time of diagnosis was 65.1 (SD 7.7) years. The proportion of males among cases was 60% and among controls 43%. Slightly more than half (58 and 51%, respectively) of cases and controls were Hokkien in dialectal group origin.

Among males, 60% (76) of cases and 56% (287) of controls had ever smoked, and 32% of both cases and controls were current smokers at baseline interview. Among females, the corresponding figures were 10 (9) and 6% (43) for ever smokers, and 7 and 5% for current smokers, respectively. Overall, there was no association between a history of smoking and colorectal cancer risk (OR 0.99, 95% CI 0.69–1.43), nor interaction between GST genotype and dietary ITC such that GSTM1 and GSTT1 null genotypes was 0.31, 95% CI 0.12–0.84) and not seen among those with rectal cancers. The OR for GSTP1 null genotype was 1.22 (95% CI 0.90–1.67), relative to GSTM1 non-null, and that for the GSTT1 null genotype was 0.88 (95% CI 0.64–1.21) relative to GSTT1 non-null genotype. Results were similar for both colon and rectal cancers. The OR for GSTP1 AB and BB genotypes, relative to the high activity AA genotype, were 0.94 (95% CI 0.67–1.33) and 0.54 (95% CI 0.20–1.41).

No significant associations were observed, and odds ratios were fairly uniform across strata, when dietary ITC intake was examined among subjects grouped by GSTM1 or GSTP1 genotype (Table III). There was a slight difference between GSTT1 null and non-null individuals. Among GSTT1 null subjects, the OR for high versus low ITC intake was 0.63 (95% CI 0.37–1.07) compared with an OR of 0.97 (95% CI 0.64–1.47) among GSTT1 non-null subjects. However, among individuals null for both GSTM1 and T1, high dietary ITC conferred a 57% reduction in risk which was statistically significant (OR 0.43, 95% CI 0.20–0.96). There were too few cases (n = 9) who were null for GSTM1 and T1, and GSTP1 AB/BB genotypes for meaningful analysis. On further analysis, this effect was confined to the subjects with colon cancer (OR 0.31, 95% CI 0.12–0.84) and not seen among those with rectal cancers (Table IV).

We also examined the effect of duration of follow-up on the ITC-colorectal cancer association. When analyses were restricted to colorectal cancers with >3 years of follow-up (n = 20), the OR for high versus low ITC intake among subjects with both GSTM1 and GSTT1 null genotypes was similar to that derived from the entire dataset (data not shown).

Discussion

In summary, in this Asian population with high cruciferous vegetable intake and colorectal cancer rates, we observe an interaction between GST genotype and dietary ITC such that high dietary ITC is associated with a significantly lower risk of colorectal cancer among individuals who are both GSTM1 and T1-null, and hence metabolize and excrete these compounds at a slower rate. The association is not seen in those who are positive for one or both of these metabolic enzymes.
In an earlier study among Singapore Chinese, Lee et al. (33) demonstrated a significant inverse association between cruciferous vegetable intake and colorectal cancer (OR 0.48, 95% 0.23–1.01 for highest versus lowest tertile, \( P \) for trend < 0.05). Among the dietary factors examined, this was the most consistent effect observed in that study. The mean intake
of eight cruciferous vegetables at the time of that study (1985–1987) was 62.5 g/day among males and 67.7 g/day among female controls. When cruciferous vegetable intake (nine varieties) was assessed for the current study approximately one decade later, the mean intakes were 42.1 and 43.4 g/day, respectively. Although study methodology was not uniform, the data suggest a fall in the intake of cruciferous vegetables that appears to parallel rising rates of colorectal cancer in the Singapore population.

While there is now a body of evidence that supports the association between cruciferous vegetables and colon cancer (1,2), the present study provides new information on the effect of GST, the main metabolic enzymes, on this relationship, and is the first to demonstrate this using ITC values calculated from the full range of cruciferous vegetables consumed in the population. Our results are in agreement with those of Lin et al. (34) who reported a protective effect of broccoli against colorectal adenomas only among GSTM1 null individuals. While the highest quartile of broccoli intake was itself protective (OR 0.47, 95% CI 0.30–0.73), among those who were also GSTM1 null, the OR was 0.36 (0.19–0.68), compared with 0.74 (0.40–0.99) (P for interaction 0.01) in those who were GSTM1 non-null. Slattery et al. (35) found that among US men and women aged 55 years and younger, risk of colon cancer decreased with increasing levels of cruciferous vegetable, and broccoli intake, and this effect was most marked among those with the GSTM1 null genotype. Among this group, the odds ratio for four or more servings per week versus no intake was 0.23 (95% CI 0.10–0.54).

There has been some uncertainty as to whether the protective effects of cruciferous vegetables can be attributed to individual compounds like ITC or indoles, or if they are due to the action of other unknown chemicals (5). The present study suggests that ITCs are indeed the major constituents in cruciferous vegetables that account for their chemopreventive activity in the colon and elsewhere. We also show that the ITC-colon cancer effect is strongest among individuals deficient for GST, the major metabolic pathway for elimination of ITCs. Similar relationships between GST, ITC and lung cancer have been demonstrated in diverse populations (36–38). Taken together, these results provide strong evidence that the inverse ITC–cancer relationship is a causal one.

The chemopreventive activity of ITCs through their ability to inhibit phase I enzymes and induce phase II enzymes in various target tissues has been demonstrated in vivo in relation to chemically induced tumorigenesis (5,8,39,40). In addition, ITCs may act as anticarcinogens through more than one pathway. Recent studies have shown that ITCs and other phase II enzyme inducers can also act as ‘suppressing agents’ during the post-initiation stage of carcinogenesis by promoting apoptosis, and suppressing malignant transformation, possibly through their effect on the cellular glutathione pool (19,41,42). Such induction of apoptosis has been demonstrated in colon cancer cell lines, and colonic crypts of dimethylhydrazine (DMH) treated rats (41,43). On the other hand, some studies have reported that benzyl ITC may increase resistance to apoptosis and promote carcinogenesis when administered post-initiation (44).

Since sufficient intracellular concentrations are required for a compound to exert its chemopreventive effect, the colon is a site where ITC chemoprevention is particularly significant. Data exist to suggest that concentrations of ITC shown to be active as enzyme inducers in vitro can be achieved in the colonic mucosa from an average serving of cruciferous vegetable such as broccoli (9,41), and this is consistent with human feeding studies, which observe altered metabolizing enzyme activity in humans given diets rich in cruciferae (5).

A fuller understanding of the metabolic processes operating in the colon requires involvement of specific colon carcinogens (45), and in this regard the possible risk-enhancing effect of meat, particularly meat cooked at high temperatures, has been of interest (46,47). In a controlled feeding study (48), ingestion of cruciferous vegetables increased conjugated urinary mutagenicity among volunteers consuming a fried meat diet, whereas ingestion of non-cruciferous vegetables did not have this effect. This increase was 2-fold higher among GSTM1 null subjects relative to GSTM1 non-null, consistent with data from the present study, and other epidemiologic studies on GST-ITC interaction.

We did not observe an independent effect of GSTM1, T1 null, or P1 AB/BB genotype, on risk of colorectal cancer. While a lower total GST activity in blood lymphocytes has been observed among individuals at higher risk of colon cancer (49), and the GSTM1 and T1 genotypes have been related to somatic genetic changes (18) epidemiologic studies are inconsistent (19). Two studies on the relationship between GGTp1 and colorectal cancer risk reported no significant association (50,51). Similarly, there have been many studies evaluating the association between GSTM1/T1 with colorectal cancer, some supporting and others refuting this (16,17,52–54). The data from the present study do not support an independent effect of GSTM1, T1 or P1 polymorphism in the colon.

There are some issues that should be considered in evaluating GST genotype as an independent risk factor for colorectal cancer, and these may also explain the lack of consistency between studies. At the target tissue level, it is probable that biotransformation ultimately depends on a delicate balance between phase I and II enzymes. In addition, among the various isoenzymes of the GST family, each may compensate to some degree for reduced activity of another, such that the effect of individual genotypes is indiscernible. In the colon, GSTP1 is the most abundant isofrom (55), and accounts for 80% of the total activity (19). An absence of the high activity allele (A) leads to a reduction in, rather than absence of, activity, which may again be difficult to demonstrate in epidemiological studies.

The strengths of our study are that dietary information was collected prospectively using a validated questionnaire which included all major cruciferae consumed in this population, allowed computation of ITC intake, and adjustment for total energy and other relevant variables. Three major GST isoenzymes were evaluated, including GSTP1, the most common isofrom in the colon. The chief limitation is the relatively short follow-up of the cohort (the mean follow-up time per subject was 5 years). However, we intend to verify this set of novel findings when a longer duration of follow-up (with a correspondingly larger number of cases) has been achieved.

In conclusion, our results provide support for an inverse relationship between high intake of ITCs from cruciferous vegetables and colorectal cancer; and this effect is most clearly seen in GSTM1 and T1 null individuals, among whom these compounds are metabolized and excreted at a slower rate. Our results also suggest that consideration of metabolic genotypes in the investigation of risk-enhancing factors, dietary or other-
wise, in the colon, may lead to a more refined understanding of the etiology of the disease.

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Appendix 1

Cruciferous vegetables listed in the Singapore Chinese Health Study questionnaire

<table>
<thead>
<tr>
<th>Common name</th>
<th>Local name, if applicable</th>
<th>Botanical name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese white cabbage</td>
<td>Pak choi, siew pak choi</td>
<td>B. chinensis</td>
</tr>
<tr>
<td>Chinese mustard</td>
<td>Kai choi</td>
<td>B. juncea var. rugosa</td>
</tr>
<tr>
<td>Chinese flowering cabbage</td>
<td>Choi sum</td>
<td>B. chinensis var. parachinensis</td>
</tr>
<tr>
<td>Watercress</td>
<td></td>
<td>N. officinale</td>
</tr>
<tr>
<td>Chinese kale</td>
<td>Kai lan</td>
<td>B. albovaria</td>
</tr>
<tr>
<td>Celery cabbage</td>
<td>Wong nga pak</td>
<td>B. pekinensis var. cylindrical</td>
</tr>
<tr>
<td>Broccoli</td>
<td></td>
<td>B. oleracea var. italica</td>
</tr>
<tr>
<td>Cauliflower</td>
<td></td>
<td>B. oleracea var. botyris</td>
</tr>
</tbody>
</table>

Head cabbage and celery cabbage were grouped as one item in the questionnaire.