Dietary Isothiocyanates, Glutathione S-transferase -M1, -T1 Polymorphisms and Lung Cancer Risk among Chinese Women in Singapore

Bin Zhao, Adeline Seow, Edmund J. D. Lee, Wee-Teng Poh, Ming Teh, Philip Eng, Yee-Tang Wang, Wan-Cheng Tan, Mimi C. Yu, and Hsin-Peng Lee

Department of Community, Occupational and Family Medicine, Faculty of Medicine, National University of Singapore, Singapore 117597 [B. Z., A. S., H-P. L.]; Departments of Pharmacology [E. J. D. L.], Pathology [M. T. J.], and Medicine [W-C. T.], Faculty of Medicine, National University of Singapore, Singapore 119260; Departments of Pathology [W-T. P.] and Respiratory and Critical Care Medicine [P. E.], Singapore General Hospital, Singapore 169608; Department of Respiratory Medicine, Tan Tock Seng Hospital, Singapore 308433 [Y-T. W.]; and University of Southern California/Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California 90033-0800 [M. C. Y.]

Abstract

Chinese populations consume a diet relatively high in isothiocyanates (ITCs), a derivative of cruciferous vegetables known to have cancer-protective effects. This class of compounds is metabolized by the glutathione S-transferase family of enzymes, which are also involved in the detoxification of tobacco-related carcinogens such as polycyclic aromatic hydrocarbons and alkyl halides. We evaluated the association between dietary isothiocyanate intake, GSTM1 and GSTT1 polymorphisms, and lung cancer risk in 420 Chinese women: 233 histologically confirmed lung cancer patients and 187 hospital controls. Among these, 58.8% of cases and 90.3% of controls were confirmed lung cancer patients and 187 hospital controls. Higher weekly intake of ITCs (above the control median value of 53.0 µmol) reduced the risk of lung cancer to a greater extent in smokers [adjusted odds ratio (OR), 0.31; 95% confidence interval (CI), 0.10–0.98] than nonsmokers (OR, 0.70; 95% CI, 0.45–1.11). The inverse association was stronger among subjects with homozygous deletion of GSTM1 and/or GSTT1. Among nonsmokers with GSTM1-null genotype, higher intake of ITCs significantly reduced the risk of lung cancer (OR, 0.54; 95% CI, 0.30–0.95), an effect not seen among those with detectable GSTM1 (OR, 1.07; 95% CI, 0.50–2.29).

Our results, in a Chinese female population, are consistent with the hypothesis that ITC is inversely related to the risk of lung cancer, and we show that among nonsmokers this effect may be primarily confined to GST-null individuals. Conjugation and elimination of ITCs is enhanced in GST-non-null relative to null individuals, such that the GST metabolic genotype modifies the protective effect of ITCs on lung cancer development.

Introduction

Epidemiological evidence for the relationship between vegetable consumption and cancer risk is compelling, and it suggests an inverse association most marked for epithelial cancers of the respiratory and alimentary tracts (1). The relationship between Brassica vegetables and lung cancer, in particular, has been among the most consistently observed (2, 3), and this genus is distinguished by its high content of glucosinolates. These compounds are hydrolyzed to form indoles and ITCs,3 which have anticarcinogenic properties (4, 5). ITCs are among the most effective chemopreventive agents known. Their chemopreventive effect has been attributed to their ability to inhibit phase I enzymes that are responsible for the bioactivation of carcinogens and to induce phase II detoxification enzymes (6). Experimental studies in animals have demonstrated the efficacy of ITCs in inhibiting lung carcinogenesis by known carcinogens, such as polycyclic aromatic hydrocarbons and NNK (5).

Human GSTs are phase II enzymes that play a major role in the detoxification of many reactive electrophilic compounds by conjugation with glutathione and also by noncovalent binding of many xenobiotics (7). GSTs can be classified into at least four genetically distinct groups (8) including GSTM1 and GSTT1. Polymorphisms in the GSTM1 and GSTT1 genes in DNA isolated from peripheral blood, which are distinguished by its high content of glucosinolates. These compounds are hydrolyzed to form indoles and ITCs,3 which have anticarcinogenic properties (4, 5).

We are grateful to the National Medical Research Council, Singapore for financial support of this study (Research Grant NMRC 1996/0155).

3 The abbreviations used are: ITC, isothiocyanate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; GST, glutathione S-transferase; OR, odds ratio; CI, confidence interval; ETS, environmental tobacco smoke.

Received 4/20/01; revised 7/19/01; accepted 8/16/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This study was funded by Research Grant NMRC 1996/0155 from the National Medical Research Council, Singapore. Dr. Mimi Yu is supported by United States National Cancer Institute Grant R35 CA58500.

2 To whom requests for reprints should be addressed, at Department of Community, Occupational and Family Medicine, Faculty of Medicine, The National University of Singapore, MD5, 15 Medical Drive, Singapore 117597. Email: cofseow@nus.edu.sg.

The abbreviations used are: ITC, isothiocyanate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; GST, glutathione S-transferase; OR, odds ratio; CI, confidence interval; ETS, environmental tobacco smoke.
the formation of the corresponding N-acetylcyesteine conjugates (dithiocarbamates) and aids in the elimination of ITCs (15). Hence the GSTs promote the elimination not only of carcinogens, but also of ITCs themselves (16), and could thus decrease ITC chemopreventive effects. Modification of the ITC-mediated protective effect in lung cancer by GSTM1 and GSTT1 polymorphisms is biologically plausible and has been reported in two recent epidemiological studies, Refs. 17 and 18, among Shanghai Chinese men, and United States whites, respectively.

Lung cancer is currently the third most commonly diagnosed cancer among Singapore Chinese women and constitutes 9.8% of all cancers in this population (19). This population is unique in having an incidence of lung cancer comparable with many countries in the West despite a smoking prevalence of only 3% (20). It is also characterized by a high intake of cruciferous vegetables; the mean intake frequency being 363 times a year (and the average amount 42.5 g/day) among Chinese women. We previously demonstrated, in the same population, that individuals with GSTT1-null genotype had significantly higher levels of urinary ITCs when stratified by dietary intake of ITCs or cruciferous, suggesting that GSTT1 is a key enzyme in the conjugation and subsequent excretion of these compounds (21). In the present study, we determined the intake of ITCs obtained by dietary questionnaire from 420 Chinese women (233 lung cancer cases and 187 controls), and we used PCR-based methods to determine their GSTM1 and GSTT1 genotypes. We examined the relationship between total ITC intake and lung cancer risk in Chinese women and the effect of GSTM1 and GSTT1 polymorphisms on this risk.

Subjects and Methods

Between April 1996 and September 1998, we conducted a case-control study on lung cancer and environmental exposures among Chinese women, details of which have been described elsewhere (22). Briefly, cases were incident lung cancers diagnosed at three of the major hospitals in Singapore. Controls were patients admitted to the same hospital as the cases, frequency-matched for age (within the same 10-year age group), with no history of cancer or any chronic respiratory condition. They were drawn from internal medicine, orthopedic, surgical/truma, and eye wards. Between January 1997 and September 1998, all participants were asked to provide 6 ml of blood by venepuncture. A total of 233 patients with pathologically confirmed primary lung cancer and 187 age-matched controls consented and were thus included in the present study. They were similar to the larger study population in terms of age, country of birth, dialect group (indicating provincial origin in China) and smoking status.

Demographic information and data on smoking were obtained by standardized questionnaire administered in-person by a research nurse, who interviewed both cases and controls equally. For cases, interviews took place within 3 months of diagnosis of cancer. Interviewers were not blind to case or control status, but possible observer bias was monitored by tape-recording and review of a random sample of interviews. Subjects were classified as smokers if they had ever smoked at least one cigarette a day for 1 year or more. Ex-smokers were smokers who had stopped smoking for 1 year or more. Pathology specimens of all cases were reviewed and classified independently by two study pathologists; only pathologically confirmed cases with a diagnosis of squamous cell carcinoma, small cell carcinoma, adenocarcinoma, or large cell carcinoma were included.

Dietary Data. Forty-five food items including fruits and vegetables were specified in the questionnaire. Of the 20 vegetables listed in the questionnaire, 9 are members of the Brassicaceae family. They are bok choi (Brassica chinensis, also known as Chinese white cabbage), kai choi (B. juncea var. rugosa, also known as mustard cabbage or Chinese mustard), kai sum (B. oleracea var. parachinensis, also known as Chinese flowering cabbage), watercress (Nasturtium officinale), kai lan (Brassica oleracea var. alboglabra, also known as Chinese kale), head cabbage (B. oleracea var. capitata), wong nga pak (B. pekinensis var. cylindrica, also known as celery cabbage), broccoflower (B. oleracea var. italica), and cauliflower (B. oleracea var. botrytis). Brussels sprouts and turnips are infrequently consumed in this population and were not included in the questionnaire. For each of these food items, the respondent was asked to indicate her average weekly serving frequency and usual serving size in the 3 years before hospital admission. Serving size was expressed as a multiple of a standard serving (standard serving = two rounded Chinese spoons of cooked vegetable). Total ITC contents in these nine cruciferous vegetables have been determined by high-performance liquid chromatography using samples obtained in Singapore (23). Estimated weekly intake of total ITCs was computed for each of the 420 study subjects via linkage of ITC contents in cruciferous vegetables with responses to the dietary questionnaire.

Identification of GSTM1 and GSTT1 Genotypes. At the time of interview, informed consent was obtained for the donation of 6 ml of blood for genotyping purposes. Isolation of genomic DNA from peripheral lymphocytes was carried out using a standard proteinase K-phenol-chloroform extraction procedure (24). A PCR method was used to detect the presence or absence of the GSTM1 and GSTT1 genes in genomic DNA samples. The absence of the GSTM1- or GSTT1-specific fragment indicated the corresponding null genotype.

The GSTM1-null genotype was determined by procedures described by Groppi et al. (25) with a slight modification. Briefly, two primers that hybridize within the fourth intron (1019: 5′-GAA GGT GGC CTC CTC CTT GG) and in the 3′ region of the fifth exon (526: 5′-AAT TCT GGA TTG TAG CAT AT) were used in the presence of another pair of primers (5′-ACA CAA CTT TGT TCA CTA GC-3′ and 5′-CTC AAA GAA CCT CTG GGT CC-3′) to amplify β-globin, included in the assay as a positive control for target DNA. A PCR reaction (amplification size: 165 bp for GSTM1 presence; 299 bp for β-globin) was performed to detect the GSTM1 deletion mutation at exon 5.

GSTT1-null genotype was determined using a similar modification of a PCR approach described previously (11), with the addition of primers for a β-globin control fragment (299 bp). The primers used to amplify the target DNA were: 5′-TTT CCT CCT ACT GGT CCT CCT ATC TC (468–491) and 5′-TCA CCG GAT CAT CCG CAG CA (703–723). The presence of at least one GSTT1 allele was identified by a 480-bp PCR product.

The presence or absence of the GSTM1 and GSTT1 genes was analyzed by ethidium bromide 1.6% agarose gel electrophoresis. All stages of the analysis were carried out blind to the patient’s disease status.

Statistical Analysis. ORs and their corresponding 95% CIs for the association between lung cancer and estimated ITC intake were computed for all subjects, ever-smokers and lifetime non-smokers. Logistic regression analysis was used to obtain age- and smoking-adjusted ORs stratified by smoking status. Intensity and duration of smoking was accounted for in the analyses
by including the number of years of smoking and the number of cigarettes smoked per day as continuous variables in the regression model. All calculations were performed using the SPSSWIN v10.0 statistical package (SPSS, Chicago, IL).

**Results**

The distribution of characteristics of the study population, which comprises 233 lung cancer patients and 187 controls is given in Table 1. Of the 420 individuals, 306 (72.9%) were lifetime nonsmokers, and 114 (26.9%) were either current or ex-smokers.

The proportion of current and ex-smokers among the cases was 41.2% (96 women), and that among the controls was 38.7% (75 women). In general, cases tended to be marginally older and there was a slight overrepresentation of Cantonese women among cases (23.2%) compared with controls (18.2%). Among the study population, cases were more likely to be foreign-born, particularly migrants from China.

The proportion of cases and controls with the GSTM1, GSTT1, and combined null genotypes was similar (Table 1). Frequencies among controls are similar to previous estimates reported for the Singapore population (26). Neither GSTM1, -T1, nor the combined null genotype was associated with lung cancer risk in this study population. The adjusted ORs (95% CIs) for the GSTM1-null versus the non-null genotypes were 1.50 (0.51–4.40) and 0.90 (0.56–1.43) for ever-smokers and lifetime nonsmokers, respectively. For GSTT1-null genotypes, the corresponding ORs were 1.95 (0.68–5.58) and 0.97 (0.62–1.53), and for the combined null genotype, they were 1.86 (0.56–6.23) and 0.95 (0.60–1.53). We did not find any significant association between GST genotype and lung cancer when the population was stratified by histological type.

Among the 420 study subjects, the distribution of estimated weekly intake level of ITCs was unimodal and markedly skewed to the right, with a range of 0.0–449.0 μmol and a median of 53.0 μmol. There was a 9.8-fold difference between the 90th and the 10th percentiles in the distribution. Table 2 shows the effect of weekly intake of ITCs on lung cancer risk. For subjects who reported an intake above the median value for controls, the risk of lung cancer was reduced. For all women, the age- and smoking-adjusted OR was 0.63 (95% CI, 0.41–0.95). The protection afforded by higher ITC intake was more marked among ever-smokers (OR, 0.31; 95% CI, 0.10–0.96) than among lifetime nonsmokers (OR, 0.70; 95% CI 0.45–1.11). Additional adjustment for place of birth did not materially affect the estimates.

Table 3 shows the effect of GSTM1 and -T1 genotypes on ITC-associated risk. Because of the small number of controls who were ever-smokers (n = 18), we present data stratified by GSTM1 and -T1 genotype for all subjects and for lifetime nonsmokers only. Among all subjects, high ITC intake conferred a 40–50% reduction in risk that was statistically significant among those with the null genotype for GSTT1, -M1, or both combined. The effect of high ITC intake was less clear among those with the non-null genotypes. Among nonsmokers, the same was true for the -M1 genotype; persons with high ITC intake and null for this genotype had a significant reduction in risk (age-adjusted OR, 0.54; 95% CI, 0.30–0.95), whereas persons with the non-null genotype did not (OR, 1.07; 95% CI, 0.50–2.29; P for interaction, 0.13). The pattern was consistent with the -T1 and combined genotypes in this subgroup as well. Overall, the magnitude of the inverse association was largest (adjusted OR, 0.47 in all subjects) among those who were both GSTM1- and GSTT1-null. In all cases, the multiplicative interaction terms for the difference in OR between null and non-null genotypes were not statistically significant.

**Discussion**

In summary, we describe an inverse association of dietary ITCs on lung cancer risk among Singapore Chinese women, which is modified by GSTM1 and -T1 genotypes. Those with the null genotype for either or both enzymes experienced a significant reduction in risk with higher intake of ITCs, but the effect was smaller and not statistically significant if either or both genes were present. We also report, for the first time, a modifying effect of the GSTM1 genotype on the effect of ITCs in lifetime nonsmokers.

Overall, our data demonstrate a significant association between dietary ITC intake and lung cancer risk. The stronger effect in smokers is not surprising, and it is consistent with the evidence that ITCs are known to reduce lung carcinogenesis by...

---

**Table 1** Distribution of selected variables among Chinese female lung cancer patients and controls [n (%)]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 233)</th>
<th>Controls (n = 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean ± SD)</td>
<td>65.5 ± 12.8</td>
<td>63.6 ± 12.0</td>
</tr>
<tr>
<td>Dialect group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hokkien</td>
<td>91 (39.1)</td>
<td>83 (44.4)</td>
</tr>
<tr>
<td>Teochew</td>
<td>58 (24.9)</td>
<td>37 (19.8)</td>
</tr>
<tr>
<td>Cantonese</td>
<td>54 (23.2)</td>
<td>34 (18.2)</td>
</tr>
<tr>
<td>Hainanese</td>
<td>18 (7.7)</td>
<td>7 (3.7)</td>
</tr>
<tr>
<td>Hakka</td>
<td>6 (2.6)</td>
<td>23 (12.3)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (2.6)</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>Country of birth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td>131 (56.2)</td>
<td>124 (66.3)</td>
</tr>
<tr>
<td>Malaysia</td>
<td>20 (8.6)</td>
<td>25 (13.4)</td>
</tr>
<tr>
<td>China</td>
<td>77 (33.0)</td>
<td>33 (17.6)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (2.1)</td>
<td>5 (2.7)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>137 (58.8)</td>
<td>169 (90.4)</td>
</tr>
<tr>
<td>Current and ex-smokers</td>
<td>96 (41.2)</td>
<td>18 (9.6)</td>
</tr>
<tr>
<td>Years of smoking (mean ± SD)</td>
<td>43.3 ± 17.7</td>
<td>39.3 ± 17.5</td>
</tr>
<tr>
<td>Number of cigarettes smoked per day (mean ± SD)</td>
<td>13.7 ± 14.6</td>
<td>10.8 ± 13.3</td>
</tr>
<tr>
<td>GSTM1-null</td>
<td>146 (62.7)</td>
<td>119 (63.6)</td>
</tr>
<tr>
<td>GSTT1-null</td>
<td>132 (56.7)</td>
<td>102 (54.5)</td>
</tr>
<tr>
<td>GSTM1- and -T1-null</td>
<td>82 (35.2)</td>
<td>66 (35.3)</td>
</tr>
</tbody>
</table>

---

**Table 2** Distribution of weekly intake level of ITCs among Chinese female lung cancer patients and controls [n (%)]

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 233)</th>
<th>Controls (n = 187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤53.0 μmol</td>
<td>132 (56.7)</td>
<td>78 (41.7)</td>
</tr>
<tr>
<td>&gt;53.0 μmol</td>
<td>101 (43.3)</td>
<td>109 (58.3)</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤53.0 μmol</td>
<td>70 (51.1)</td>
<td>72 (42.6)</td>
</tr>
<tr>
<td>&gt;53.0 μmol</td>
<td>67 (48.9)</td>
<td>97 (57.4)</td>
</tr>
<tr>
<td>Current and ex-smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤53.0 μmol</td>
<td>62 (64.4)</td>
<td>6 (33.3)</td>
</tr>
<tr>
<td>&gt;53.0 μmol</td>
<td>34 (35.4)</td>
<td>12 (66.7)</td>
</tr>
</tbody>
</table>

* Adjusted for age (in completed years).
* Further adjusted for smoking. Smoking-related regression covariates were smoking at recruitment (yes/no), years of smoking, and number of cigarettes smoked per day.

---
Polycyclic aromatic hydrocarbons such as benzo(a)pyrene and NNK, a tobacco-specific nitrosamine, require metabolic activation. Agents such as ITC, which decrease formation of the electrophilic DNA binding intermediates, reduce DNA damage and thereby inhibit carcinogenesis. Mechanistic studies have shown that this chemopreventive activity is attributable to the inhibition of phase I enzymes and the induction of phase II enzymes (6). Specifically, phenethyl ITC has been shown to inhibit NNK-induced lung tumorigenesis in animal studies (27, 28), and the consumption of watercress by smoking volunteers led to increased urinary excretion of NNK metabolites (5).

The most thoroughly studied examples of ITC inhibition of carcinogenesis are in relation to tobacco-related carcinogens. Tobacco-specific nitrosamines, require metabolic activation. Agents such as ITC, which decrease formation of the electrophilic DNA binding intermediates, reduce DNA damage and thereby inhibit carcinogenesis. Mechanistic studies have shown that this chemopreventive activity is attributable to the inhibition of phase I enzymes and the induction of phase II enzymes (6). Specifically, phenethyl ITC has been shown to inhibit NNK-induced lung tumorigenesis in animal studies (27, 28), and the consumption of watercress by smoking volunteers led to increased urinary excretion of NNK metabolites (5).

The most thoroughly studied examples of ITC inhibition of carcinogenesis are in relation to tobacco-related carcinogens (29), and the evidence linking ITC to lung cancer risk among nonsmokers is less consistent than for smokers. However, the experimental evidence does point to the capability of ITCs to inhibit carcinogenesis in a wide range of target organs and against a variety of chemical carcinogens (29). Among the nonsmokers is less consistent than for smokers. However, the experimental evidence does point to the capability of ITCs to inhibit carcinogenesis in a wide range of target organs and against a variety of chemical carcinogens (29).

Mechanistic studies have shown that this chemopreventive activity is attributable to the inhibition of phase I enzymes and the induction of phase II enzymes (6). Specifically, phenethyl ITC has been shown to inhibit NNK-induced lung tumorigenesis in animal studies (27, 28), and the consumption of watercress by smoking volunteers led to increased urinary excretion of NNK metabolites (5).

The most thoroughly studied examples of ITC inhibition of carcinogenesis are in relation to tobacco-related carcinogens (29), and the evidence linking ITC to lung cancer risk among nonsmokers is less consistent than for smokers. However, the experimental evidence does point to the capability of ITCs to inhibit carcinogenesis in a wide range of target organs and against a variety of chemical carcinogens (29). Among the nonsmokers is less consistent than for smokers. However, the experimental evidence does point to the capability of ITCs to inhibit carcinogenesis in a wide range of target organs and against a variety of chemical carcinogens (29).
ITCs from cruciferous vegetable consumption protect against lung cancer, and we extend these findings to a Chinese population with a high proportion of lifetime nonsmokers. In addition, ITC intake and GSTM1 and GSTT1 polymorphisms interact in the etiology of lung cancer such that persons with the null genotype experience a greater reduction in risk because these compounds are less rapidly metabolized and eliminated from the body.

Acknowledgments

We are grateful to the Medical Boards of the National University Hospital, Singapore General Hospital, and Tan Tock Seng Hospital, Singapore, for permission to carry out this study at their institutions. We also thank Dr. Yap Wai Ming for his kind help in facilitating the pathological review.

References

Dietary Isothiocyanates, Glutathione S-transferase -M1, -T1 Polymorphisms and Lung Cancer Risk among Chinese Women in Singapore

Bin Zhao, Adeline Seow, Edmund J. D. Lee, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/10/10/1063

Cited articles
This article cites 25 articles, 8 of which you can access for free at:
http://cebp.aacrjournals.org/content/10/10/1063.full#ref-list-1

Citing articles
This article has been cited by 26 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/10/10/1063.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cebp.aacrjournals.org/content/10/10/1063.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.