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Chemopreventive Potential of Thiol Conjugates of Isothiocyanates for Lung Cancer and a Urinary Biomarker of Dietary Isothiocyanates

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Abstract Natural and synthetic isothiocyanates (ITCs) are versatile chemopreventive agents in many animal systems. We have shown that phenethyl ITC (PEITC) and 6-phenylhexyl ITC (PHITC) are potent inhibitors against lung tumorigenesis induced by tobacco nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in both mouse and rat. The mechanism by which these ITCs inhibited lung tumorigenesis is attributed to their ability to decrease cytochrome P450 (P450) enzyme activities involved in the activation of NNK. Recently, we have found that thiol conjugates of ITCs inhibit P450 enzymes and are effective inhibitors of lung tumorigenesis. This is significant because conjugation with cellular thiols is the major route of ITC metabolism via the mercapturic acid pathway in rodents and humans. The thiol conjugates are less pungent and potentially less toxic, and they are more soluble and chemically less reactive than ITCs. These properties raise the prospect of substituting thiol conjugates for ITCs as chemopreventive agents. Furthermore, although ample rodent studies have established that ITCs inhibit tumorigenesis, the protective role of dietary ITCs against human cancers has not yet been established. As a prerequisite for such human studies, we have developed an HPLC-based assay, based on the condensation reaction of ITCs or conjugates with 1,2-benzenedithiol, for measuring a cyclocondensation product in human urine as an uptake biomarker of total ITCs. This assay was validated using urine samples from subjects who had ingested a known amount of watercress or mustard in a controlled diet. The assay is convenient and rapid, showing promise for analyzing urine samples obtained from population-based studies. Results from two such studies are presented to illustrate the potential application of this biomarker in epidemiologic studies. J. Cell. Biochem. Suppl. 27:76–85.

Key words: thiol conjugates; isothiocyanates; lung cancer prevention; urinary biomarker

A significant amount of work carried out in laboratory animals has clearly shown that isothiocyanates (ITCs) are efficacious chemopreventive agents for tumorigenesis induced by a variety of carcinogens at a number of organ sites. ITCs inhibit lung, esophageal, and pancreatic tumor development in rodents treated with nitrosamines, and block forestomach, liver, lung, and mammary tumorigenesis by heterocyclic amines and polycyclicaromatic hydrocarbons (PAHs) [1]. The inhibitory potency of ITCs depends on the structures, the carcinogen used, and the species and target organ examined. We have shown that phenethyl ITC (PEITC) and 6-phenylhexyl ITC (PHITC) are highly effective in preventing lung tumor development in mice and rats treated with the potent nicotine-derived nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) [2,3]. The homologue, benzyl ITC (BITC), however, is not inhibitory toward NNK-induced lung tumorigenesis in A/J mice, but blocks benzo(a)pyrene (B(a)P)-induced lung and forestomach tumorigenesis [2,4,5]. Similarly, PEITC effectively blocks esophageal tumor development in rats treated with *N*-nitrosomethylbenzylamine, whereas its longer chain homologues, 4-phenylbutyl ITC (PBITC) and PHITC, are inactive [6]. Both in vitro and in vivo studies have shown

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that the tumor inhibitory activity of ITCs can be attributed to their ability to inhibit cytochrome P450s (P450s) and/or to induce phase II enzymes involved in activation or detoxification of carcinogens [7,8].

ITCs are electrophiles which react predominantly with thiols, and, to a much lesser degree, with NH_2 and OH groups, resulting in covalent binding with cellular proteins [9]. The binding of ITCs to P450 proteins is, at least in part, responsible for their inhibitory activity [10]. Consistent with the facile reaction of ITCs with proteins, the conjugation with glutathione (GSH) constitutes a major route of ITC metabolism. Our studies have demonstrated that more than 50% of PEITC and allyl ITC (AITC) administered through watercress and mustard consumption, respectively, is excreted as the *N*-acetylcysteine (NAC) conjugate in human urine [11,12]. The conjugation reactions of ITCs with GSH are catalyzed by human glutathione transferases (GSTs) [13,14]. Conjugated ITCs are then degraded through the mercapturic acid pathway and excreted in urine as NAC conjugates. The toxicity of ITCs has been well documented, depending on dose and structure. For examples, BITC and phenyl ITC (PITC) are toxic in A/J mice at 25 µmol oral dose, but PEITC is not [2]. A 13-week toxicity study has shown that PEITC given at a concentration of 15.3 mmol/kg in the diet to F344 rats causes morphological alterations in the epithelial lining of the forestomach. However, at 3 mmol/kg, an effective chemopreventive dose, PEITC does not elicit toxic effects (Stoner GD, personal communication). Chronic treatment with a high dose of AITC induces urinary bladder tumors in rats [15]. ITCs are cytotoxic and, as a result, some ITCs have been shown to inhibit the growth of tumor cells in culture [16,17]. BITC possesses antibacterial activity and has been used as a clinical drug in Germany [18]. Recent studies have shown that PHITC, a remarkably efficacious inhibitor of NNK lung tumorigenesis, enhances tumorigenicity in esophagus, skin, and colon in rats treated with carcinogen [4,19,20]. It is speculated that the enhancement of tumorigenesis by ITCs is, in part, due to their chronic toxicity toward specific tissues. Furthermore, acute cecal hemorrhage has been observed in rats treated with PHITC; however, this appears to be reversible (Chung FL and Rivenson A, unpublished results). Recently, our studies have shown that the thiol conjugates of ITC inhibit metabolism of NNK and *N*-nitrosodimethylamine (NDMA) (P450 2E1) by rat liver microsomes [21]. They also inhibit P450 2B1 1A1 and 1A2 [22]. Prompted by these findings, we examined the thiol conjugates in an A/J mouse lung tumorigenesis bioassay. In this paper, we describe these recent findings and discuss their potential importance.

Although studies in rodents have shown that ITCs and their conjugates are effective and versatile inhibitors of tumorigenesis, an important question remains: does dietary intake of ITCs play a role in lowering cancer risk among people who frequently consume cruciferous vegetables? A prerequisite to epidemiological studies designed to answer this question is to develop uptake biomarkers of ITCs in humans. In this study, we describe an HPLC-based assay to quantify total ITCs in human urine. This assay was developed based on a previously described reaction of ITCs or ITC conjugates with 1,2-benzenedithiol to form a cyclocondensation product [23]. We further validated this biomarker using urine samples collected from subjects who had eaten meals containing a known watercress or brown mustard. The potential application of this biomarker was examined using urine samples accrued from two cohort studies, one in Shanghai, China, and one in Singapore. Both populations are known to have relatively high consumption of cruciferous vegetables.

RESULTS

Inhibition of Rodent and Human Hepatic P450 Isozyme Activity by ITCs of Various Structures and Their Conjugates

The inhibition of P450 2E1 in rat liver microsomes was determined by a radiometric *N*-nitrosodimethylamine demethylase (NDMAd) assay [24]. For P450 2B1 and P450 1A1 and 1A2, the dealkylations of pentoxyresorufin (PROD) and ethoxyresorufin (EROD) were assayed [25,26]. The structures of ITCs examined are shown in Figure 1. The IC_{50} values of ITCs and their conjugates toward these P450 isozymes are summarized in Figure 2. Sulforaphone (SFO), a major ITC in broccoli and a potent phase II enzyme inducer in hepatoma cell culture [27], was not significantly inhibitory toward these enzyme activities. Similarly, AITC, abundant in brown mustard and also a phase II enzyme inducer [28,29], showed very weak inhibitory activity. In contrast, BITC and PEITC significantly inhibited P450 2E1 and

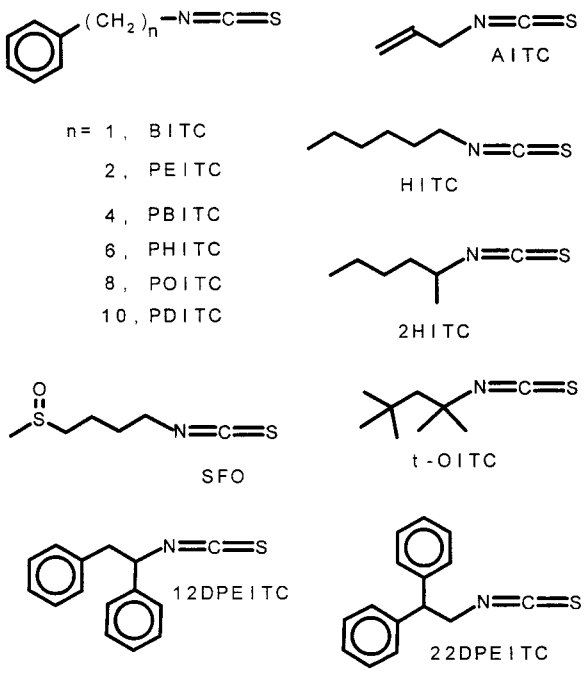

Fig. 1. Structures of ITC examined.

2B1 activity in rat liver microsomes. Interestingly, GSH conjugates of BITC and PEITC were at least as potent as their parent ITCs in the P450 2E1 assay, but were less potent than the parent ITCs in the P450 2B1 assay. ITCs and their conjugates were much more inhibitory toward P450 2E1 and 2B1 than P450 1A1 and 1A2. Among the conjugates of PEITC, PEITC-cysteine (Cys) and PEITC-GSH were both potent inhibitors of NDMAd activity, whereas PEITC-NAC was much less active. Analogous results were observed with these conjugates toward P450 2B1.

To examine the ability of ITCs and their conjugates to inhibit human P450 2E1 activity, we assayed PEITC and its conjugates toward NDMAd in human liver microsomes. The inhibition curves for the selected compounds, together with Cys, GSH and NAC as controls, were plotted in Figure 3. While Cys, GSH, and NAC showed little effect on human P450 2E1 activity within the concentration range tested, PEITC and its conjugates significantly inhibited the activity in a dose-dependent manner. Consistent with results obtained from rat liver microsomes, PEITC, PEITC-Cys and PEITC-GSH had similar IC_{50} values (6–9 μM), whereas PEITC-NAC was much less potent (85 μM) [21].

For both PROD and EROD a clear correlation of the inhibitory potency with chain length of the ITCs was observed. The pattern extended from C_1 (BITC) up to C_6 (PHITC), and then declined with further increase of chain length (C_8 to C_{10}). An increase in potency with increasing alkyl chain length has been previously reported when arylalkyl ITCs (C_1–C_6) were assayed as competitive inhibitors of NNK metabolism, using microsomes prepared from A/J mouse lung, F344 rat lung, and rat nasal mucosa [30]. The phenyl ring does not appear essential for inhibition of P450 isozymes, since octylisothiocyanate (*t*-OITC), hexylisothiocyanate (HITC), and 2-HITC, which do not contain the phenyl ring, were relatively strong inhibitors of both PROD and EROD. 1,2-Diphenylethyl ITC (1,2-DPEITC), 2,2-DPEITC, and 2,2-DPEITC-GSH, which possess an additional phenyl ring on the PEITC structure, were much stronger inhibitors than PEITC and PEITC-GSH, respectively, for both PROD and EROD. The potencies of 1,2-DPEITC, 2,2-DPEITC, and 2,2-DPEITC-GSH as inhibitors for P450 1A1 and 1A2 was among the highest for all structures examined in this study, suggesting that the active site on P450 1A1 is shaped to harbor compounds with multiple phenyl groups and that these di-substituted phenyl ITCs are potential inhibitors of B(a)P-induced tumorigenesis.

Inhibition of NNK Metabolism in Mouse Lung Microsomes and NNK Lung Tumorigenesis by ITCs and Their Conjugates

The effects of PEITC and PHITC conjugates, together with their parent ITCs, on NNK metabolism were studied in mouse lung microsomes. The rates of formation for the three major oxidative metabolites of NNK, keto aldehyde, keto alcohol, and NNK-N-oxide, were analyzed. The inhibitory potency of the compounds tested, as indicated by their IC_{50} values for these metabolic pathways of NNK, is summarized in Table I. As shown in our previous studies, both PEITC and PHITC strongly inhibited the formation of all three NNK metabolites, and PHITC was considerably more inhibitory than PEITC [31]. PEITC-GSH and PEITC-Cys were also inhibitory, but were less potent than PEITC. PEITC-NAC was only weakly inhibitory under the incubation conditions. The order of inhibitory potency of PEITC and its conjugates is PEITC $>$ PEITC-GSH $>$ PEITC-Cys $\gg$ PEITC-NAC. An increased inhibitory potency with increasing alkyl chain

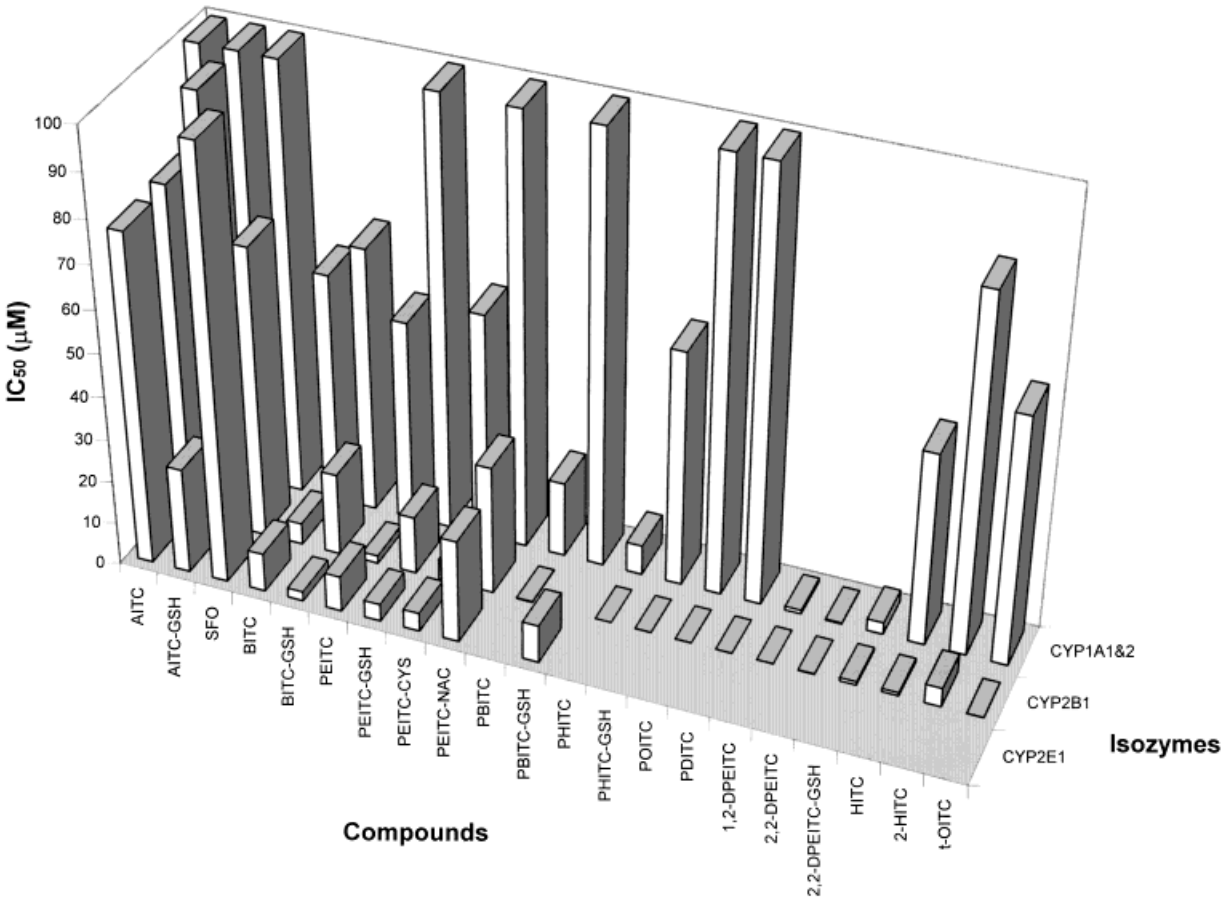

Fig. 2. IC_{50} values of various ITCs and conjugates toward P450 2E, 2B1, and 1A1 and 1A2 in rat liver microsomes. A radiometric NDMA demethylase assay was used for P450 2E1 [26]. The dealkylation of ethoxyresorufin (EROD) and pentoxyresorufin (PROD) was assayed for P450 1A1 and 2 and P450 2B1, respectively [25,26].

length was observed among the GSH conjugates of BITC, PEITC, and PHITC.

The results of in vitro metabolism prompted us to examine the potential of ITC conjugates in the lung tumorigenesis bioassay in A/J mice treated with a single dose of NNK. The tumor multiplicity and incidence are summarized in Figure 4. NNK-treated mice pretreated with vehicle alone developed 16.5 tumors/mouse and a 100% incidence. Mice in the control group without NNK treatment developed only 0.2 tumor/mouse with a 20% incidence. These background tumor responses are similar to those reported previously [32,33]. At a single dose of 5 μmol, PEITC inhibited tumor multiplicity by 28%. Although the percentage of inhibition is less than previously reported at the same dose, this is a significant reduction compared with the NNK treated control group (group 1, $P < 0.01$). PEITC-GSH at 4 μmol did not inhibit lung tumorigenesis. However, at 8 μmol, it reduced tumor multiplicity by 32% ($P < 0.01$). Although it had no effect on tumor incidence, PEITC-NAC at both dose levels, 5 and 20 μmol/mouse, inhibited about 30% of tumor multiplicity. We did not observe a dose-dependent inhibition by PEITC-NAC. In contrast, PHITC-NAC showed a remarkable inhibitory effect and a clear dose-related inhibition of tumor multiplicity and incidence. It reduced the tumor multiplicity from 16.5 to 5.0 at 1 μmol and completely blocked the NNK-induced lung tumorigenesis at 5 μmol; only background tumors were observed at this dose.

Our previous tumor bioassays have shown that the arylalkyl ITCs are potent inhibitors of NNK-induced lung tumorigenesis in the A/J mouse; the potency increased with increasing chain length up to C_6 [3,32,33]. Further, an increase in the alkyl chain length to C_8 or C_{10} as in 8-phenyloctyl ITC (POITC) and 10-phenyldecyl ITC (PDITC) did not significantly im-

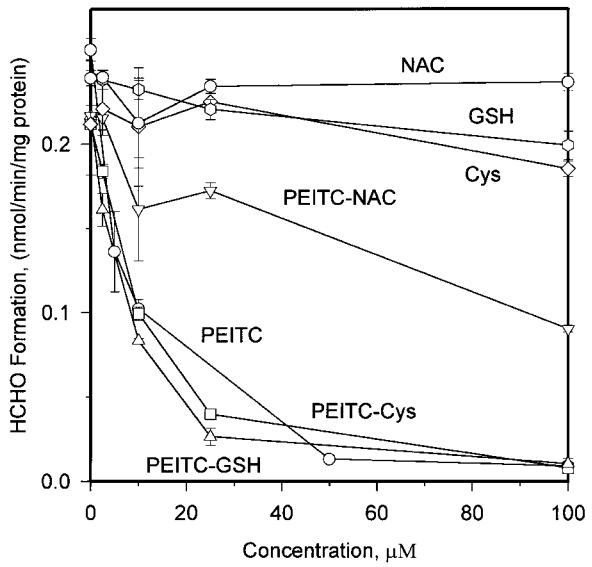

Fig. 3. Dose-dependent inhibition of NDMA demethylation activity in human liver microsomes by PEITC and its conjugates. Each data point represents the average of three incubations. The incubation mixture (0.1 mL) contains 97 μg of human liver microsomal protein suspended in a pH 7.4 Tris buffer, and NADPH (1–100 μM). Blanks were done without adding NADPH-generating system, and controls were done without adding inhibitors. Incubation was carried out at 37°C for 20 min. While Cys, NAC, and GSH had little effect on the NDMA demethylase activity, PEITC and its conjugates inhibited the enzyme activity with IC_{50} values: 6 μM (PEITC), 9 μM (PEITC-Cys), 8 μM (PEITC-GSH), and 85 μM (PEITC-NAC) [21].

TABLE I. Inhibition of NNK Metabolism in Mouse Lung Microsomes by ITCs and Conjugates*

	IC_{50} (nM)		
Inhibitors	Keto aldehyde	Keto alcohol	NNK-N-oxide
PEITC	500	450	400
PEITC-GSH	680	725	720
PEITC-Cys	1,000	1,280	960
PEITC-NAC[a]	—	—	—
BITC-GSH	780	1,750	1,800
PHITC	65	55	40
PHITC-GSH	75	50	50
PHITC-NAC	55	45	40

*Incubation mixtures contained 10 μM NNK (containing 1 μCi [5-^{3}H]NNK), an NADPH-generating system, 5 mM sodium bisulfite, 0.1 mg microsomal protein, and 0–5 μM ITC or conjugates (8 concentrations). The tested compounds were dissolved in a phosphate buffer (pH 7.4), except for PEITC, PEITC-Cys, and PHITC, which were dissolved in methanol. Incubations were carried out for 20 min at 37°C. Each experiment was in duplicate and the average values were used. Data were plotted as rate of formation vs. the concentration of the inhibitor and the value of IC_{50} was obtained from the plot by using graphic interpolation. All inhibitor solutions were prepared and used fresh.

[a]PEITC-NAC did not inhibit the oxidation of NNK by 50% at the concentrations used in the assay. At 5 μM, the oxidative metabolism of NNK was only inhibited by 16–19%.

prove the inhibitory activity as compared to its C_6 homologue, PHITC [33]. Introducing a phenyl ring to PEITC, as in 1,2-DPEITC and 2,2-DPEITC, enhanced its potency to the degree similar to PHITC. Both naturally occurring alkyl ITCs, AITC and SFO (Chung and Jiao, unpublished results), showed no inhibitor activity against NNK-induced lung tumorigenesis [33]. The close agreement of inhibition toward PROD with tumor inhibitory activity supports the theory that P450 2B1 plays an important role in activation of NNK in A/J mouse, [7] and suggests that P450 2B1 is a key target for the chemopreventive action of isothiocyanates.

Development of Urinary Biomarker of Dietary ITCs

Previously, we described assays for measuring specific NAC conjugates of PEITC or AITC in human urine after consumption of watercress or brown mustard, respectively [11,12]. In the present study, we developed a convenient HPLC-based assay, based on the condensation reaction of 1,2-benzenedithiol with ITCs described by Zhang and Talalay [8], to quantify the cyclic condensation product in human urine as an uptake biomarker for total dietary ITCs. Cyclic condensation reaction also occurs with ITC conjugates due to the reversible reaction of dithiocarbamates which releases ITCs [13,34]. To validate this biomarker, we analyzed 14 urine samples containing NAC conjugate of PEITC or AITC collected from individuals who had ingested a known amount of watercress or brown mustard [12,35]. Both assays generated small standard variations, indicating good reproducibility in triplicate experiments for each urine sample and the reliability of the HPLC-based analyses. We obtained a correlation coefficient of $r = 0.978$ between the two sets of data from these assays (Fig. 5).

To test the applicability of this biomarker to urine specimens obtained from epidemiologic studies, we analyzed urine samples collected from two studies, a cohort study in Shanghai, China, and a cohort study of Chinese in Singapore. These two populations are frequent con-

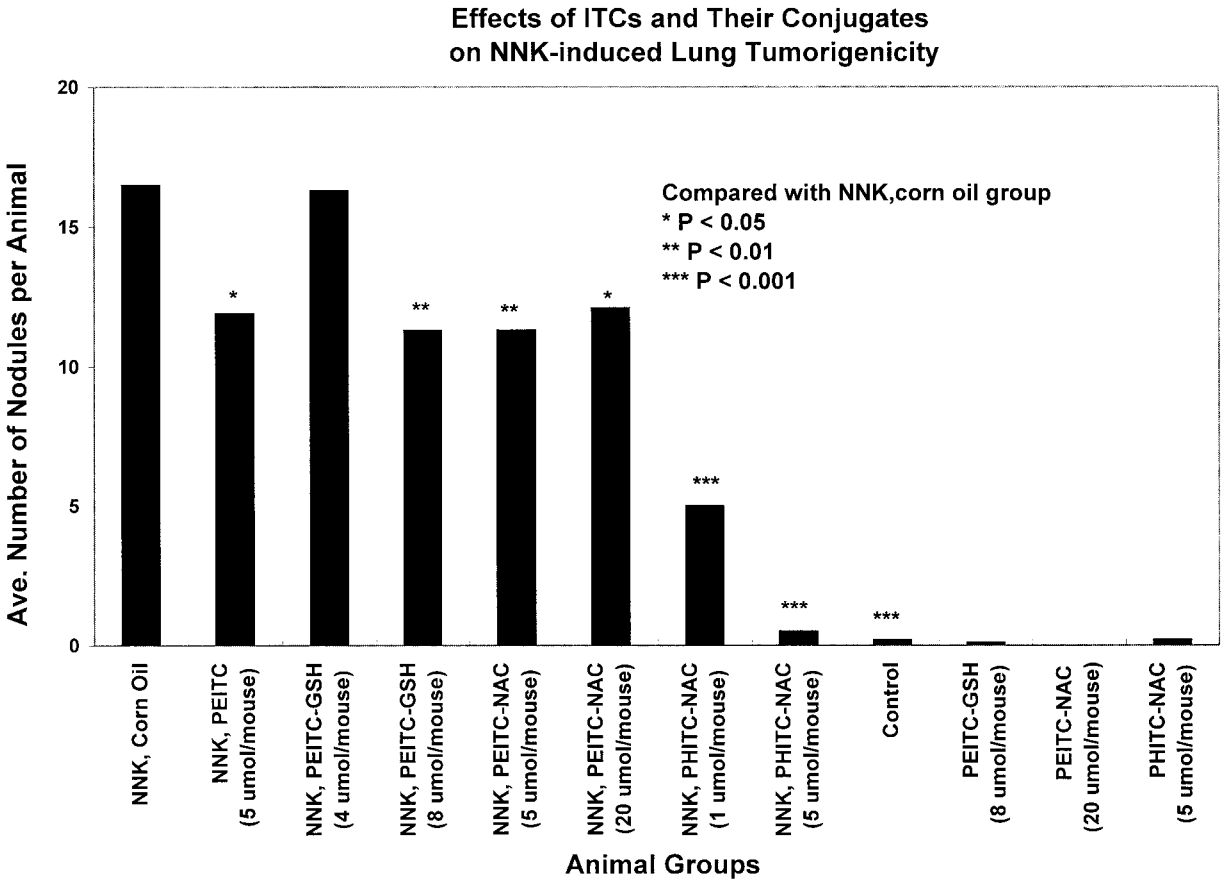

Fig. 4. Inhibition of lung tumorigenesis by ITC conjugates in NNK-treated A/J mice. The single-dose protocol for NNK lung tumor induction in A/J mouse was used [34]. In brief, 30 mice were each given a single dose of 10 µmol NNK (in 0.1 mL saline) i.p., serving as the NNK control group. Groups of 15 or 18 mice were treated with test agents by gavage in 0.1 ml vehicle as solution or suspension at the dose of 1–20 µmol. Two hours after treatment with test agents, mice were administered 10 µmol NNK (in 0.1 mL saline) by i.p. injection. Mice in the control groups were treated with either corn oil alone or test agents in corn oil, followed by i.p. injection of saline. The body weight in each group was measured at the beginning of the assay and then once every month until termination. Sixteen weeks after the NNK or saline administration, mice were sacrificed and pulmonary adenomas were quantified. Tumor multiplicities were compared using analysis of variance (ANOVA) followed by Dunnett's procedure.

sumers of green vegetables. As a pilot study, only nine urine samples collected during 1986 to 1989 from the Shanghai study were analyzed [36]. All nine subjects had indicated, during baseline interviews, that they were daily consumers of dark green vegetables. Although all the urine samples were shown to contain ITC, we found relatively low amounts of ITCs or dithiocarbamates in these samples, as compared to samples collected from individuals who consumed a controlled diet containing known amounts of brown mustard or watercress. Table II shows that a wide range of concentration, from 0.3 to 11 µM, was found in these samples. After correction with creatinine, a 10-fold range was still evident among the samples. A total of 246 samples collected during 1993–1996 from the Singapore study were assayed. These specimens represent random samples of the cohort subjects recruited during the specific time period. The data are summarized in Figure 6, and indicate a wide distribution of urine ITC concentrations in this population.

DISCUSSION

Our studies show that CYP 2E1, 1A1 and 2, and 2B1 are inhibited by ITC conjugates in rat liver and human liver microsomes [21,22]. We also demonstrated that the conjugates of ITCs inhibit NNK metabolism by mouse lung microsomes and NNK-induced lung tumorigenesis in A/J mice. We reported that in the series of GSH conjugates of BITC, PEITC, and PHITC, the

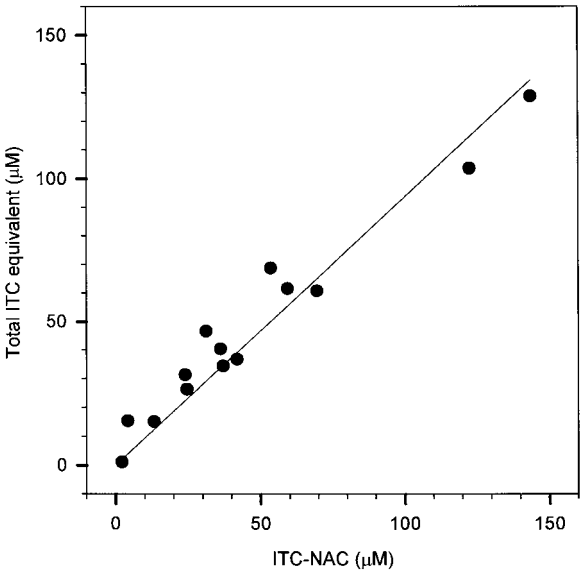

Fig. 5. Pearson correlation coefficient (r = 0.978) of specific ITC conjugates and cyclocondensation product in 14 urine samples collected from individuals who had consumed a known amount of watercress or brown mustard in a controlled diet [11,35].

TABLE II. Urinary Concentrations of ITCs and Creatinine in Nine Daily Consumers of Dark Green Vegetables From a Cohort Study in Shanghai, China, Accrued During 1986–1989 [36]

Sample no.	ITC (μM)[a]	Creatinine (mg/ml)	ITC equivalent/ creatinine (μmol/g)
01582	0.3 ± 0.1	0.403	0.7
01912	0.6 ± 0.1	0.256	2.3
04981	0.3 ± 0.1	0.454	0.7
06436	11.0 ± 1.0	1.573	7.0
06473	0.6 ± 0.1	0.603	1.0
14187	4.0 ± 0.6	1.125	3.6
14558	1.5 ± 0.7	0.835	1.8
15719	4.8 ± 0.8	1.346	3.6
17684	1.3 ± 0.3	1.633	0.8

[a]Mean ± S.D. from triplicate measurements.

inhibitory potency increases with increasing alkyl chain length in ITCs, a trend parallel to that observed with the parent ITC. This result suggests that a common mechanism of inhibition is shared by both ITCs and their conjugates. Based on the labile nature of the dithiocarbamate linkage in the conjugates and their ability to dissociate to free ITCs in aqueous solution, it is likely that ITCs are the ultimate active species responsible for inhibition. The

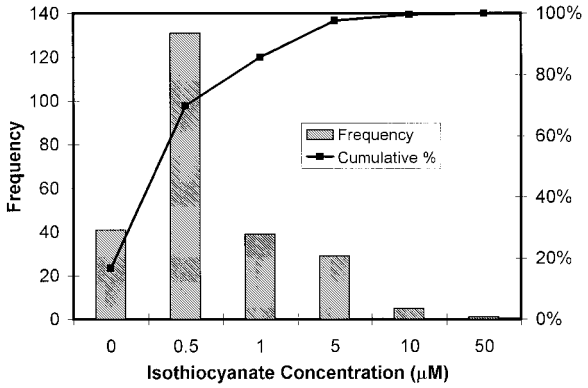

Fig. 6. Histogram of data obtained from analysis of total ITC content in 246 randomly selected urine samples from a cohort study in Singapore.

IC_{50} values for conjugates of PEITC also appear to support this theory. PEITC-NAC is a much less potent inhibitor in NNK metabolism than PEITC-GSH and PEITC-Cys. Our previous study showed that PEITC-NAC has the longest half-life in the ligand exchange reaction with free thiol groups in buffer solution and, therefore, the slowest dissociation rate among the three conjugates [21]. This could explain the low inhibitory activity of PEITC-NAC under in vitro assay conditions. However, we did not observe the same trend in PHITC conjugates. PHITC-GSH and PHITC-NAC appeared as potent as PHITC itself. We cannot completely rule out the possibility that the intact conjugate may also be an inhibitor. The direct inhibition of enzymatic activity by the conjugates may possibly explain the observed near-equal potency between PHITC and its conjugates, but would not account for the trend with PEITC conjugates. Apparently, further studies are needed to delineate the detailed mechanism of inhibition of P450s by the conjugates.

We demonstrated that ITC conjugates are inhibitors of lung tumorigenesis. Several properties make ITC conjugates potentially more desirable than ITCs as chemopreventive agents. They are more soluble and less reactive, and are considerably less pungent than ITC. As the depots of ITCs, the conjugates provide a slow release of ITCs and consequently are likely to be less toxic than the parent ITCs. Therefore, demonstration of anticarcinogenic activity of ITC conjugates is an important step in developing more effective chemopreventive agents as substitutes for ITCs. It has been reported that

GSH and L-Cys conjugates of BITC are less toxic than BITC in RL-4 rat hepatocytes [34]. In A/J mice, L-Cys conjugate of BITC and 3-phenylpropyl ITC are less toxic than parent ITCs at a high dose (100 μmol/mouse) [37]. The gradual release of ITCs in tissue via deconjugation could be the reason for the reduced toxicity.

NAC conjugates of BITC, PEITC, and AITC are the major metabolites found in human urine after consumption of gardencress, watercress, and brown mustard [11,12,18]. Our previous studies have shown an intake-dependent excretion of these conjugates in urine, suggesting their utility as biomarkers of exposure to specific ITC in diet [11,12]. However, the application of these biomarkers in population-based studies is limited to selected people whose diets contain vegetables rich in these ITCs. A more useful biomarker, which has more general application, would reflect total dietary ITC intake. The urinary biomarker developed in this study, although lacking specificity for individual ITC, provides better detectability than specific ITC mercapturic acid metabolites. This is illustrated in our attempts to analyze the allyl or phenethyl ITC conjugate in urine samples obtained from the Shanghai cohort study. We were unable to detect these conjugates in urine samples obtained from this study, probably due to infrequent consumption of vegetables rich in these specific ITCs. Other important features of this assay are its speed and high reproducibility (<5% intra-assay variability). The assay requires no sample extractions and involves only an initial centrifugation of urine samples and reaction with 1,2-benzenedithiol, followed by a second centrifugation before HPLC analysis.

The cyclocondensation reaction of 1,2-benzenedithiol also occurs with a number of thionyl compounds such as thiourea, carbon disulfide, and disulfiram [23]. Although it is unlikely that these compounds could interfere with the assay, we cannot completely rule out the possibility. Several compounds often present in human urine, such as nitrile, thiocyanate, and isocyanate, did not form the condensation product. Furthermore, the specificity of the assay for ITCs and their conjugates is supported by results from the study using urine samples collected from individuals who had consumed a watercress or mustard diet. Although this sample size is small, the close agreement between the values of the specific conjugates and the biomarker provides evidence that the cyclic thionyl derivative comes primarily from mercapturic acids of ITCs in the urine. Since reaction conditions used in the assay were established to quantitatively convert ITC conjugates to the cyclic product, we believe that the amounts detected accurately reflect the total urinary content of ITC conjugates.

CONCLUSION

We observed a wide range of total ITCs in urine samples accrued in the cohort studies in Shanghai, China, and Singapore. These results suggest that the cyclocondensation product is a potential biomarker for dietary ITCs. This assay is feasible for samples stored for up to 10 years. Rodent studies suggest that ITCs may be one of the active ingredients in cruciferous vegetables responsible for their protective effect against cancer. Together with results from laboratory studies, application of the biomarker described here in population-based studies may provide important information regarding the role of these compounds in protecting against human cancers.

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