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Effects of 2-phenethyl isothiocyanate on metabolism of 1,3-butadiene in smokers

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Abstract

2-Phenethyl isothiocyanate (PEITC) is a natural product found as a conjugate in cruciferous vegetables. It has been reported to have preventative properties against lung cancer and to inhibit metabolic activation of tobacco carcinogens. In the present study, we evaluated the ability of PEITC to influence the metabolism of the human carcinogen 1,3-butadiene in current smokers in a phase II clinical trial with a crossover design. Urinary mercapturic acids of 1,3-butadiene were quantified at baseline and during PEITC treatment. Seventy-nine smokers were randomly assigned to one of two arms: PEITC followed by placebo, or placebo followed by PEITC. During the 1-week treatment period, each subject took PEITC (10 mg in 1 mL of olive oil, 4 times per day). There was a 1-week washout period between the PEITC and placebo periods. Oral ingestion of PEITC increased urinary levels of BD-mercapturic acids (MHBMA and DHBMA) by 11.1% and 3.7%, respectively, but these increases were not statistically significant ($p = 0.17$ and 0.64, respectively). A much stronger effect was observed among subjects with the null genotype of both *GSTM1* and *GSTT1*: in these individuals, PEITC increased urinary levels of MHBMA by 58.7% ($p = 0.004$) and 90.0% ($p = 0.001$), respectively, but did not have a significant effect on urinary DHBMA. These results reveal a potentially protective effect of PEITC treatment with respect to the detoxification of 1,3-butadiene in cigarette smokers, specifically in those null for *GSTT1*, and provide further evidence in support of stronger chemopreventive effects from consumption of dietary isothiocyanates in these individuals.

Keywords

2-Phenethyl isothiocyanate; 1,3-butadiene; glutathione-*S*-transferase; lung cancer; chemoprevention; clinical trial

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Introduction

Isothiocyanates are a class of naturally occurring compounds found in fruits and vegetables that have been shown to have chemopreventive effects through multiple mechanisms of action (1,2). One such compound, 2-phenethyl isothiocyanate (PEITC, Figure 1), found in watercress, garden cress, radishes, and turnips (3), modulates multiple cancer-associated pathways, such as cell cycle arrest (4–8), NFκB (9,10), and apoptosis (8,11). PEITC can inhibit cytochrome P450 monoxygenases (CYP450s) involved in metabolic activation of carcinogens (12) and induce detoxifying enzymes such as glutathione-*S*-transferases (GSTs) (13). More specifically, treatment with PEITC inhibited lung carcinogenesis in laboratory mice and rats exposed to 4-(methylnitrosamino)-1-(3-pyridyl)-1- butanone (NNK), a potent tobacco-specific carcinogen (14–16). PEITC ingestion by current smokers has also been shown to decrease the metabolic activation of NNK to DNA-reactive species (17).

Although the link between smoking and lung cancer is well established, smoking remains the most preventable cause of death in the world (18). Worldwide, there are approximately 1.1 billion smokers (19), with 80% and 50% of lung cancer deaths attributed to tobacco use in men and women, respectively (20). Among the 40 million smokers in the United States, cigarette smoking causes 84% of total lung cancer deaths in men and 79% of all lung cancer deaths in women (18,19). While smoking cessation is the best method of reducing smoking related lung cancer deaths, inhibition of the carcinogenic and genotoxic effects of cigarette smoke remains an important alternative route in the fight against smoking related deaths. The use of chemopreventive compounds naturally occurring in various food sources could provide a widely available and relatively inexpensive method of cancer prevention.

Recently, the effects of PEITC on the metabolism of cigarette smoke carcinogens and toxicants including benzene, acrolein, and crotonaldehyde were investigated by measuring their respective mercapturic acids, detoxification products formed through glutathione (GSH) conjugation via glutathione-S-transferases (GSTs) (21). A statistically significant increase in urinary concentrations of mercapturic acids formed from benzene and acrolein was observed when smokers were given PEITC, while no such increase was seen in urinary concentrations of the respective mercapturic acid formed from crotonaldehyde in all smokers (21). The effects of PEITC were more substantial in individuals null for GSTs mu 1 and theta 1 (*GSTM1* and *GSTT1*); for these subjects, PEITC treatment significantly increased urinary concentrations of the mercapturic acids of benzene, acrolein, and crotonaldehyde, indicating that use of isothiocyanates could provide enhanced protection against lung carcinogenesis in individuals lacking these genes (21).

In this study, we investigated the effects of PEITC consumption on the metabolism of 1,3-butadiene (BD) in smokers. BD (Figure 1) is among one of the most abundant carcinogens present in cigarette smoke likely to contribute to the etiology of lung cancer,(22) with concentrations of 20–75 μg and 205–360 μg per cigarette in mainstream and sidestream smoke, respectively (23,24). Besides tobacco smoke, humans are exposed to BD in occupational settings in the production of synthetic rubber and polymers (25). BD has been classified as a known human carcinogen by the National Toxicology Program (NTP) and as a Group 1 agent by the International Agency for the Research on Cancer (IARC) (26,27).

Occupational exposure to BD is associated with the development of leukemia and lymphoma, (25,28–34). The ability of BD to modify DNA bases depends on cytochrome P450-mediated formation of reactive metabolites such as 3,4-epoxy-1-butene (EB) and hydroxymethylvinyl ketone (HMVK). EB and HMVK undergo detoxification through conjugation with glutathione, a reaction catalyzed by GSTs, to ultimately form the mercapturic acids 1- and 2-(*N*-acetyl-L-cysteine-S-yl)-1-hydroxybut-3-ene (MHBMA) and *N*-acetyl-*S*-(3,4-dihydroxybutyl)-L-cysteine (DHBMA), respectively (Figure 1) (35–38). GSTs play an important role in the detoxification of BD epoxides (39–42). Oral supplementation with PEITC has been shown to facilitate glutathione conjugation of benzene and acrolein in individuals with *GSTT1* and *GSTM1* gene deletion (17). However, the effects of PEITC on the metabolic detoxification of BD have not been previously investigated.

Here, we employed quantitative HPLC-ESI$^-$-MS/MS methods previously developed in our laboratory (41,43) to measure urinary concentrations of BD-mercapturic acids MHBMA and DHBMA (Figure 1) (35), in smokers supplemented with PEITC. Oral ingestion of PEITC induced statistically significant increases in urinary MHBMA concentrations in smokers lacking the *GSTT1* gene or both the *GSTT1* and *GSTM1* genes. These results are consistent with epidemiological studies revealing a stronger protection by dietary PEITC in individuals lacking both *GSTT1* and *GSTM1* (44).

Experimental

Materials

LC-MS grade H_2O, methanol, and acetonitrile were acquired from Fisher Scientific (Pittsburgh, PA). All other chemicals and solvents were obtained from Sigma-Aldrich (St. Louis, MO). MHBMA, DHBMA, $^2H_6$-MHBMA, and $^2H_7$-DHBMA were purchased from Toronto Research Chemicals (Toronto, Canada). Oasis HLB 96 well plates were procured from Waters Corp. (Milford, MA).

Study Design

The study was a randomized, placebo-controlled, double-blind, phase II clinical trial with a crossover study design (Figure 2). Details of the study design can be found in prior publications (17,21). Over a duration of five weeks, qualified participants were asked to smoke cigarettes containing [pyridine-D_4]NNK to allow for measurement of NNK metabolism. After an adjustment period of two weeks, individuals were placed into one of two treatment groups: those receiving PEITC before receiving a placebo or those receiving a placebo before receiving PEITC. PEITC (10 mg in 1 mL olive oil) was administered 4 times per day every 4 hours for 5 days during weeks three or five; the placebo (1 mL olive oil) was administered on the same schedule, either during weeks three or five. Week four consisted of a washout period, where participants did not receive PEITC or the placebo. This study was approved by the Institutional Review Boards of the University of Minnesota (0712M22651) and the University of Pittsburgh (PRO11110669). Patient studies were conducted in accordance with U.S. Common Rule ethical guidelines, and informed written consent was obtained from all subjects.

Twenty-four hour urine samples were collected at the end of weeks two and four and on days three, four, and five of weeks three and five. For the purposes of this investigation, urine samples used for analysis were from weeks two and four and day five of weeks three and five. Total nicotine equivalents and total NNAL were quantified using high-throughput liquid chromatography-MS/MS assays described previously (37). Blood and buccal cell samples were collected at the end of each week. DNA from blood lymphocytes collected during week one was used to genotype *GSTM1* and *GSTT1*. Details regarding genotyping methods can be found in prior publications (17,21,45).

HPLC-ESI⁻-MS/MS Analysis of Urinary MHBMA and DHBMA

Urinary concentrations of BD-mercapturic acids (MHBMA and DHBMA) were determined by isotope dilution HPLC-ESI⁻-MS/MS as described in our earlier publications (41–43). The method's limits of detection (LOD) were 0.2 ng/mL urine and 5 ng/mL urine for MHBMA and DHBMA, respectively. One sample was discarded for having DHBMA values below the LOD of the method. Quality control (QC) samples were included three times per batch, fifteen times total, for the purposes of quality control and to account for any inter-batch variation. The mean coefficient of variation for these replicates was 11.0% and 11.8% for MHBMA and DHBMA, respectively.

Statistical Analyses

Urinary MHBMA and DHBMA concentrations were adjusted to creatinine by dividing each value by the appropriate creatinine value and by batch. The average value of the creatinine-adjusted outcomes of each batch were taken to get $a_{1, \ldots, a_5}$ and calculated $\overline{a} = \frac{a1 + \ldots + a5}{5}$. To adjust for those in batch one, each MHBMA and DHBMA value was multiplied by $\overline{a}$ and divided by a_1. Similar processes were done for subjects in batches two through five. Therefore, final outcomes were defined as below:

$$MHBMA\ outcome\ for\ set\ i = \frac{MHBMA}{Cr}\frac{\overline{a}}{a_i}$$

These outcomes were log-transformed and back-transformed and presented as geometric means. In addition to adjusting for creatinine and batch, the models were adjusted by log-transformed creatinine-adjusted TNE (total nicotine equivalents). Eight subjects were removed from all analyses due to missing outcomes at baseline. One subject was removed from all analyses because the urinary DHBMA concentration was below the LOD of the method.

Baseline demographics and urinary biomarkers were summarized using means and standard deviations for continuous variables, frequencies and percentages for categorical variables, and geometric means and 95% confidence intervals for urinary biomarkers (Table 1). To determine if there were associations between variables at baseline and treatment sequence, Chi-square or Fisher's exact tests were used for categorical variables, when appropriate, and Student's t-tests were used for continuous variables.

To determine whether there was an effect of PEITC on the urinary MHBMA or DHBMA concentrations, linear mixed models with random effects that also take into account period and sequence effects were used. Similar models were used to determine if there was an effect of PEITC treatment when stratified by GST genotype. An interaction term between treatment and GST genotype was also included to investigate if GST genotype modifies the effect of PEITC on the urinary MHBMA or DHBMA concentrations. Due to log-transformation of the outcomes, geometric means and percentage change are presented.

To investigate the relationship between GST genotype and the outcomes at baseline, a linear regression model was used with a covariate for GST genotype, and adjustment for creatinine-adjusted TNE. All reported p-values are two-sided and a significance level of 0.05 was used. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina).

Results

Characteristics of the study participants are detailed in Table 1. Between the two randomly assigned treatment sequences, e.g. PEITC-Placebo and Placebo-PEITC, there was no significant difference in age, body mass index (BMI), gender, race, level of education, amount of cigarettes smoked per day (CPD), or alcohol consumption. Of the 79 total participants, 53% were men, 67% were white, 22% were black, and the overall mean age was 41.2 years, with an average CPD smoked of 21.6. There were 7.6% of study participants with the *GSTT1* null genotype, 30.4% with the *GSTM1* null genotype, and 15.2% with the double null genotype. Overall, baseline levels of urinary MHBMA and DHBMA were not statistically significantly different between the two treatment groups (Table 1); there was a nearly significant difference between the two sequences for TNE ($p = 0.05$), which was still adjusted for in the later analyses.

We next examined the effects of PEITC treatment on urinary MHBMA and DHBMA in smokers. Comparison of PEITC treatment effect versus that of the placebo on urinary concentrations of MHBMA and DHBMA is summarized in Table 2. When compared to the placebo, urinary concentrations of both MHBMA and DHBMA increased with PEITC treatment (12.27 vs. 11.04 ng MHBMA/mg Cr and 593.81 vs. 572.91 ng DHBMA/mg Cr), but this increase was not statistically significant ($p = 0.17$ and 0.64, respectively).

A much stronger effect was observed when data was stratified by *GSTM1* and *GSTT1* genotype (Table 3). In participants null for *GSTT1*, PEITC increased urinary MHBMA levels by 58.7% as compared to the placebo (6.93 vs. 4.37 ng/mg Cr; $p = 0.004$). In participants null for both *GSTT1* and *GSTM1*, PEITC treatment increased urinary MHBMA levels by 90.0% as compared to the placebo (5.0 vs. 2.6 ng/mg Cr; $p = 0.001$). PEITC treatment also resulted in a 19.5% increase of urinary MHBMA in individuals lacking *GSTM1,* although these differences were not statistically significant ($p = 0.12$). *GSTT1* and *GSTM1* genotype did not significantly influence PEITC treatment effect on urinary DHBMA, with the interaction terms for *GSTT1*, *GSTM1*, and both *GSTT1*/*GSTM1* with treatment group being $p = 0.67$, 0.82, and 0.33, respectively (Table 3). Overall, these results indicate that oral ingestion of PEITC by smokers shifts BD metabolism towards

detoxification via conjugation with glutathione, with more pronounced relative effects observed in individuals with null genotypes of *GSTT1* alone or both *GSTT1* and *GSTTM1.*

A weaker effect was observed for DHBMA, with limited influence of PEITC treatment on urinary metabolite concentrations (Table 3). Previous studies conducted in several laboratories indicate that DHBMA is only weakly associated with smoking and may have an endogenous source (37,43). Furthermore, urinary DHBMA levels were not affected by genetic polymorphisms in *GSTT1* or other xenobiotic metabolism genes (41,42). Based on these observations, MHBMA appears to be a better biomarker of inter-individual differences in butadiene metabolism, despite its lower concentrations in human urine (37).

In untreated individuals at week two baseline, smokers with at least one copy of *GSTT1* excreted 219.5% more MHBMA than in those with null *GSTT1* genotype ($p = 0.01$, Table 4). In contrast, *GSTM1* genotype did not have a significant effect on urinary MHBMA concentrations in smokers. These data support results from our previous publications showing the influence of *GSTT1* genotype on BD metabolism to MHBMA (41–43). Furthermore, neither *GSTT1* nor *GSTM1* genotype appeared to have a significant effect on baseline levels of urinary DHBMA ($p = 0.22$ and 0.24, respectively, Table 4), though concentrations were higher in individuals null for either gene as compared to individuals with at least one copy. Participants null for *GSTT1* excreted 891.3 ng DHBMA/mg Cr versus 645.9 ng/mg Cr in individuals with the gene present (Table 4); participants null for *GSTM1* excreted 803.4 ng DHBMA/mg Cr versus 615.8 ng/mg Cr in individuals with the gene present (Table 4).

Discussion

The chemopreventive properties of isothiocyanates are well documented, showing inhibition of carcinogenesis at multiple sites in rodents including mammary gland (46,47), lung (16,48–53), pancreatic (54), colon (55,56), skin (57), and liver tissues (58). In particular, PEITC has been found to attenuate carcinogenesis caused by a variety of chemical carcinogens including 7,12-dimethylbenz[*a*]anthracene (DBMA) (46), *N*-nitrosobenzylmethylamine (NBMA) (59,60), azoxymethane (AOM) (55,56), and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (16,48,49).

Our results presented herein reveal that treatment with PEITC increases urinary concentrations of both MHBMA and DHBMA in smokers by 11.1% and 3.7% respectively; however, these increases were not statistically significant (Table 2). Further differentiation of the data by genotype, however, revealed that PEITC treatment significantly increased urinary MHBMA levels by 56.7% in individuals null for *GSTT1* (Table 4). Given that individuals null for *GSTT1* had the lowest baseline levels of urinary MHBMA (7.8 ng/mg Cr, Table 4), the results indicate that treatment with PEITC could provide a protective effect in those null for this genotype, possibly inducing other GSTs to catalyze detoxification of BD derived epoxides. Our previous work has shown that GSTT2 is able to catalyze the formation of an EB-GSH conjugate, the precursor to MHBMA, but further studies are warranted to determine the importance of this particular protein in metabolic detoxification of BD-derived reactive species (41). These findings provide additional support to prior work that shows the

marked effect of *GSTT1*-null status on urinary MHBMA levels (41–43), and are similar to a recent study examining the effects of *GSTT1*-null status on mercapturic acids formed from metabolites of other chemical carcinogens, such as benzene, acrolein, and crotonaldehyde (21).

The effect of *GSTM1* genotype on urinary MHBMA however, was less distinct. PEITC treatment increased MHBMA levels independent of *GSTM1* genotype, and although individuals null for the gene saw a greater increase (19.5%) than those containing the gene (5.2%), the difference between treatment with PEITC and the placebo was not statistically significant (Table 4). Furthermore, there was no significant difference in baseline levels of MHBMA between individuals null for *GSTM1* versus those where the gene was present ($p = 0.67$, Table 4); however, this could be due to a smaller sample size, as our previous study involving a much larger number of subjects (n = 1, 068 versus 79) did show significant differences among smokers when stratified by *GSTM1* genotype (41). In that work, individuals with two, one, or no copies of the gene excreted urinary MHBMA concentrations of 5.5, 5.3, and 4.4 ng/mL urine, respectively ($p < 0.0001$) (41). Interestingly, the largest increase in urinary MHBMA by PEITC treatment was seen in individuals null for both *GSTT1* and *GSTM1* (89.97%, Table 4), still potentially indicating a protective effect, albeit small, in *GSTM1*-null individuals.

Unlike MHBMA, PEITC treatment did not have a significant effect on urinary DHBMA concentrations, even when stratified by GST genotype. However, prior work also indicates that GST genotype does not necessarily seem to influence excretion of this particular mercapturic acid. In the same aforementioned study containing 1,068 subjects, statistical analysis showed no significant effect on urinary DHBMA in smokers when data was stratified by *GSTT1* or *GSTM1* copy number ($p = 0.226$ and 0.94, respectively) (41); in an additional study containing 584 subjects, analyses also showed no significant effect on urinary DHBMA in smokers ($p = 0.181$) (42). These results could likely be due to the fact that DHBMA is typically a less sensitive biomarker of BD exposure from smoking as compared to MHBMA. In a smoking cessation study, urinary levels of MHBMA decreased 92% three days post cessation, whereas DHBMA levels only decreased 16% in the same amount of time; this disparity remained throughout the study (37). Additionally, urinary DHBMA in smokers is only about 35% higher than in nonsmokers, suggesting potential DHBMA formation from sources other than BD (37,61).

Because treatment with PEITC lasted only one week, there are potential limitations that accompany the interpretation of this data. Effects of long term treatment with PEITC on the urinary concentrations of mercapturic acids formed from carcinogen metabolites remainsto be evaluated; further investigation is warranted as PEITC itself can form conjugates with GSH (62), potentially acting as a scavenger of free GSH. Still, data from animal studies in which the effects of long term PEITC treatment in laboratory rodents were examined do support its use as chemopreventive agent. In multiple published studies (15,63), PEITC exhibited the ability to inhibit lung tumorogenesis: rats given PEITC in drinking water were completely protected from lung carcinogenesis induced by the tobacco specific carcinogen NNK in an 111 week-long study (15). NCr nude mice injected with colon cancer (SW260) cells previously treated with PEITC (2.5 μM) for six weeks showed delayed tumor growth

and significantly decreased tumor weight compared to controls (64). *In vitro* work attributed these results to the ability of long term PEITC treatment to decrease DNA methylation at known anti-cancer gene loci, suggesting that this type of treatment could induce stable epigenetic modifications in tumor cells (64). Additional *in vivo* work in F344 rats compared the effect of PEITC treatment (as 0.1% of the animals' diet) during initiation of multi-organ carcinogenesis over a period of four weeks to PEITC treatment post-initiation over a period of 22 weeks, and found that treatment during the initiation phase showed inhibitory effects of carcinogenesis in esophageal, kidney and liver tissues, whereas treatment during the post-initiation phase showed inhibitory effects in lung tissue (65). However, to our knowledge, there are no post-exposure studies demonstrating the long-term effects of PEITC after treatment is terminated.

Overall, this study is the first of its kind to examine the effect of PEITC treatment on BD metabolism in smokers. Our results suggest that ingestion of PEITC could provide a strongly protective effect against BD-mediated carcinogenesis in smokers null for *GSTT1* or both *GSTT1* and *GSTM1*. More broadly, these results support other work investigating the anticancer properties of dietary isothiocyanates and provide additional evidence that consumption of these compounds could provide a wide-reaching and cost effective method of cancer prevention.

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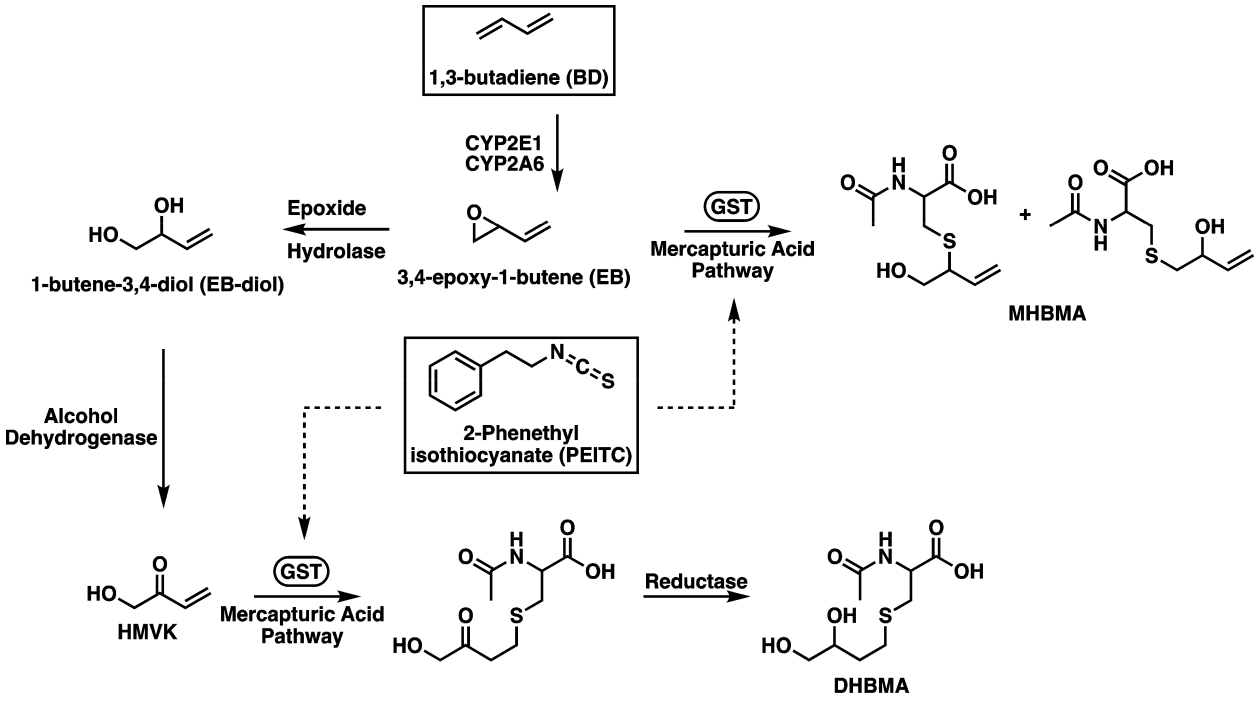

Figure 1.

Schematic showing the metabolism of BD; dotted lines represent induction of GSTs by PEITC.

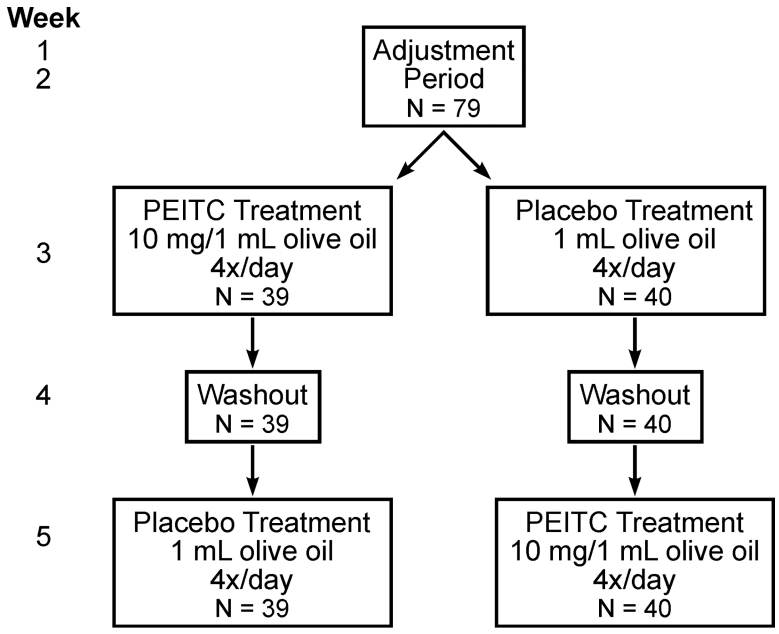

Figure 2.
Flow Diagram Outlining Study Design

Table 1.

Summary of study demographics and urinary biomarkers at baseline.

	Treatment Sequence Assignment		
Characteristics or Biomarkers	**PEITC-Placebo**	**Placebo-PEITC**	**P-value[a]**
Number of subjects[b]	39	40	
Age (years), mean (SD)	41.6 (10.5)	40.8 (9.6)	0.74
Body mass index (kg/m^2), mean (SD)[d]	28.2 (4.8)	28.2 (6.3)	0.97
Gender, n (%)			0.31
Male	23 (59.0)	19 (47.5)	
Female	16 (41.0)	21 (52.5)	
Race, n (%)			>0.99
Black	8 (20.5)	9 (22.5)	
White	26 (66.7)	27 (67.5)	
Other	5 (12.8)	4 (10.0)	
Level of education, n (%)			0.20
High school or lower	13 (33.3)	19 (47.5)	
College or higher	26 (66.7)	21 (52.5)	
Cigarettes per day, mean (SD)[e]	22.2 (9.4)	21.1 (7.1)	0.57
Alcohol drinking, n (%)[d]			0.69
Never	13 (34.2)	17 (43.6)	
Monthly or less	13 (34.2)	12 (30.8)	
Weekly	12 (31.6)	10 (25.6)	
GSTM1 and *GSTT1* genotypes, n (%)			0.67
Present and present	19 (48.7)	18 (45.0)	
Present and null	3 (7.7)	3 (7.5)	
Null and present	13 (33.3)	11 (27.5)	
Null and null	4 (10.3)	8 (20.0)	
Urinary biomarkers[c], geometric mean (95% CI)			
Total nicotine equivalents (TNE, nmol/mg Cr)	45.7 (38.1, 54.8)	59.3 (48.9, 71.8)	0.05
MHBMA (ng/mg Cr)	15.2 (10.5, 22.1)	13.4 (9.9, 18.3)	0.60
DHBMA (ng/mg Cr)	753.3 (601.2, 943.9)	642.7 (442.2, 934.1)	0.46

[a] P-value is for Student's t-test for continuous variables and Chi-Square test or Fisher's exact test for categorical variables.

[b] There were 88 subjects, but nine were removed from all analyses: eight due to missing outcomes at baseline, and one due to values of DHBMA and LOD.

[c] Urinary biomarkers are adjusted for creatinine and log-transformed. MHBMA and DHBMA were also adjusted for batch.

[d] Two subjects were excluded from this analysis due to missing data.

[e] Five subjects were excluded from this analysis due to missing data.

Table 2.

Urinary MHBMA and DHBMA concentrations in smokers treated with PEITC and placebo.

	Geometric means			
Urinary biomarkers[a]	**Placebo**	**PEITC**	**% Difference (95% CI)**	**P-value**[b]
MHBMA (ng/mg Cr)	11.0	12.3	11.13 (−4.2, 28.9)	0.17
DHBMA (ng/mg Cr)	572.9	593.8	3.7 (−10.9, 20.6)	0.64

[a]Urinary biomarkers were adjusted for creatinine and batch, and log-transformed.

[b]Two-sided p-values were from mixed models that test the PEITC treatment effect, after adjusting for creatinine-adjusted TNE

Table 3.

Effect of PEITC compared to placebo on urinary MHBMA and DHBMA, stratified by GST genotype.

GST genotype	N[a]	Geometric mean		% Difference (95% CI)	P-value[b]	P-value[c] interaction
		Placebo	PEITC			
MHBMA (ng/mg Cr)[d]						
GSTT1						
Null	18	4.4	6.9	58.7 (17.1, 115.0)	0.004	0.01
Present	61	14.5	14.4	−0.4 (−15.24, 17.1)	0.96	
GSTM1						
Null	36	11.4	13.6	19.5 (−4.4, 49.4)	0.12	0.40
Present	43	10.7	11.3	5.2 (−13.7, 28.2)	0.62	
GSTT1 & GSTM1						
Both null	12	2.6	5.0	90.0 (30.3, 176.9)	0.001	0.01
One present	30	19.3	19.1	−1.3 (−21.6, 24.2)	0.91	
Both present	37	11.0	11.3	2.7 (−16.4, 26.0)	0.80	
DHBMA (ng/mg Cr)[d]						
GSTT1						
Null	18	713.1	696.2	−2.4 (−29.3, 34.8)	0.88	0.67
Present	61	536.6	567.9	5.8 (−10.9, 25.7)	0.52	
GSTM1						
Null	36	596.6	631.4	5.8 (−15.8, 32.9)	0.63	0.82
Present	43	553.1	565.1	2.2 (−16.7, 25.3)	0.84	
GSTT1 & GSTM1						
Both null	12	716.7	613.7	−14.4 (−42.5, 27.5)	0.45	0.33
One present	30	580.2	688.9	18.7 (−6.9, 51.5)	0.17	
Both present	37	528.0	523.1	−0.9 (−20.3, 23.2)	0.93	

[a] Value of N varies based on missing outcomes or adjusting variables.

[b] Two-sided p-values were from the mixed models that test PEITC effect on the change of MHBMA and DHBMA within each specific GST genotypes before and after PEITC intake, after adjusting for creatinine-adjusted TNE.

[c] Two sided p-values were from the mixed models that test the interaction term between PEITC intake and GST genotype on the levels of MHBMA and DHBMA, after adjusting for creatinine-adjusted TNE.

[d] Urinary biomarkers were adjusted for creatinine and batch, and log-transformed.

Table 4.

Effects of GST genotype on urinary MHBMA and DHBMA concentrations at week two baseline.

GST genotype	N	Geometric mean (95% CI)	
		MHBMA (ng/mg Cr)[a]	DHBMA (ng/mg Cr)[a]
GSTT1			
Null	18	7.8 (4.9, 12.5)	891.3 (568.8, 1396.8)
Present	61	17.1 (13.3, 22.0)	645.9 (507.5, 822.1)
% Difference		219.5 (28.3, 375.6)	72.5 (−56.6, 21.1)
P-value[b]		0.01	0.22
GSTM1			
Null	36	15.1 (10.7, 21.5)	803.4 (584.3, 1104.5)
Present	43	13.6 (9.9, 18.7)	615.8 (460.6, 823.3)
% Difference		90.0 (−44.4, 45.5)	76.7 (−50.5, 18.8)
P-value[b]		0.67	0.24

[a]Urinary biomarkers were adjusted for creatinine and batch, and log-transformed.

[b]Two-sided p-values were from linear regression models that test the GST genotype effect on week 2 baseline values, after adjusting for creatinine-adjusted TNE.