Inhibition of lung tumorigenesis in A/J mice by N-acetyl-S-(N-2-phenethylthiocarbamoyl)-L-cysteine and myo-inositol, individually and in combination

Stephen S. Hecht¹, Pramod Upadhyaya, Mingyao Wang, Robin L. Bliss, Edward J. McIntee and Patrick M. J. Kenney

University of Minnesota Cancer Center, Minneapolis, MN 55455, USA

Isothiocyanates, their N-acetylcysteine conjugates, and myo-inositol (MI) are inhibitors of lung tumorigenesis in A/J mice. However, chemoprevention by combinations of these compounds in different temporal sequences has not been examined. This is important for developing practical approaches to lung cancer chemoprevention in smokers and ex-smokers. We used a tumor model in which A/J mice are treated with 8 weekly doses of benzo[a]pyrene (B[a]P) plus 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and killed 19 weeks after the final treatment. In Experiment 1, isothiocyanates or their N-acetylcysteine conjugates were added to the diet (1 or 3 µmol/g) from 1 week before until 1 week after carcinogen treatment. The compounds were 2-phenethyl isothiocyanate (PEITC), 3-phenylpropyl isothiocyanate (PPITC), N-acetyl-S-(N-benzylthiocarbamoyl)-L-cysteine (BUTC-NAC), N-acetyl-S-(N-2-phenethylthiocarbamoyl)-L-cysteine (PEITC-NAC), and N-acetyl-S-(N-3-phenylpropylthiocarbamoyl)-L-cysteine (PPITC-NAC). Significant reductions in lung tumor multiplicity were observed in mice treated with PEITC, PEITC-NAC, PPITC and PPITC-NAC. PEITC-NAC was chosen for combination studies with MI (Experiment 2). Mice were treated with B[a]P plus NNK without or with PEITC-NAC (3 µmol/g diet), MI (55.5 µmol/g diet), or PEITC-NAC plus MI (3 µmol plus 55.5 µmol/g diet). Different temporal sequences of dietary additions were investigated: carcinogen treatment phase; post-carcinogen treatment phase; entire experiment; 50% of carcinogen treatment phase until termination; and 75% of carcinogen treatment phase until termination. All treatments reduced lung tumor multiplicity except PEITC-NAC post-carcinogen or from 75% of the carcinogen treatment phase. Reduction of lung tumor multiplicity by PEITC-NAC plus MI was greater than that in the mice treated with the agents alone in all temporal sequences. When all results were combined, PEITC-NAC plus MI was significantly more effective than the agents alone. There was a significant trend for reduction in lung tumor multiplicity with increased duration of treatment by the chemopreventive agents. These results provide a basis for further development of mixtures of PEITC-NAC and MI for chemoprevention of lung cancer.

Introduction

Lung cancer is the most common cancer in the world [1]. It is the leading cause of cancer death in the USA, with nearly 155,000 deaths expected in 2002 [2]. Cigarette smoking causes 87% of lung cancer [3]. A decline in smoking prevalence in the USA, which began after the first Surgeon General’s report in 1964, was followed by a decline in the death rate from lung cancer in the 1990s [4]. This demonstrates the power of primary prevention. However, smoking prevalence has not changed markedly since 1990 in the USA. About 25% of the adult population continued to smoke during this period. In 1999, 23.5% were smokers, amounting to 46.5 million people [5]. Ex-smokers are also at high risk for lung cancer. The relative risk is similar to that of a smoker for the first five years after quitting. It then gradually declines and reaches about that of a lifelong non-smoker 20 years after cessation [6]. There are 44.8 million ex-smokers in the USA [5]. Smokers and ex-smokers constitute high-risk groups that are logical targets for chemoprevention. However, there are presently no chemopreventive agents with proven efficacy against lung cancer in humans [7–11]. Our goal is to identify and develop such agents.

Carcinogens are the cause of lung cancer in smokers and ex-smokers [12]. Cigarette smoke contains over 60 established carcinogens [13]. The multiple genetic changes observed in human lung cancer arguably result from interactions of these metabolically activated carcinogens with DNA [14]. As there are multiple carcinogens and genetic changes involved in lung cancer etiology, it is logical that a mixture of chemopreventive agents will be necessary to retard or counteract their effects. We have been developing isothiocyanates and myo-inositol (MI) for chemoprevention of lung cancer, but these agents have never been assessed in combination [15, 16]. One goal of this study was to lay the groundwork for chemoprevention by a mixture of an isothiocyanate or its N-acetylcysteine conjugate and MI. N-Acetylcysteine conjugates of isothiocyanates (dithiocarbamates) are major mammalian isothiocyanate metabolites with established chemopreventive activity [17]. Structures of the chemopreventive agents considered in this study are shown in Figure 1.

Temporal effects are also important in chemoprevention. Wattenberg classified chemopreventive agents as blocking agents or suppressing agents [18]. Blocking agents prevent the carcinogen from reaching critical target sites such as DNA. These agents are usually tested by administration before or simultaneously with the carcinogen. Suppressing agents inhibit the evolution of the neoplastic process in cells that could become malignant. These agents are usually tested by administration after the carcinogen. Suppressing agents would generally be considered for use in ex-smokers. However, the situation with smokers is more complex. No smoker will begin using a chemopreventive agent before starting to smoke, nor is it likely that he or she will begin chemoprevention in the early part of his or her smoking years. Experimental studies

Abbreviations: B[a]P, benzo[a]pyrene; BITC, benzyl isothiocyanate; BUTC-NAC, N-acetyl-S-(N-benzylthiocarbamoyl)-L-cysteine; MI, myo-inositol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PEITC, 2-phenethyl isothiocyanate; PEITC-NAC, N-acetyl-S-(N-2-phenethylthiocarbamoyl)-L-cysteine; PPITC, 3-phenylpropyl isothiocyanate; PPITC-NAC, N-acetyl-S-(N-3-phenylpropylthiocarbamoyl)-L-cysteine
Materials and methods

**Chemicals**

2-Phenethyl isothiocyanate (PEITC) and B[a]P were obtained from Aldrich Chemical Co., Milwaukee, WI. MI was purchased from Sigma Chemical Co., St Louis, MO. 3-Phenylpropyl isothiocyanate (PPITC) was procured from LKT Laboratories, St Paul, MN. NNK was synthesized as described previously [20]. N-Acetyl-S-(N-benzylthiocarboxamoyl)-L-cysteine (BITC-NAC), N-acetyl-S-(2-phenethylthiocarboxamoyl)-L-cysteine (PEITC-NAC), and N-acetyl-S-(3-phenylpropylthiocarboxamoyl)-L-cysteine (PPITC-NAC) were synthesized according to procedures described in the literature [21,22] and structures were confirmed by 1H NMR and MS.

**A/J mouse tumorigenecity experiments**

The study design for induction of tumors by a mixture of B[a]P plus NNK was essentially the same as previously described [15,19]. Female A/J mice, 5–6 weeks of age, were obtained from The Jackson Laboratory, Bar Harbor, ME. The mice were housed in the specific pathogen-free animal quarters of the University of Minnesota Cancer Center. Upon arrival, they were maintained on AIN-93G pellet diet. When given in the diet [24]. The diets were prepared every 4 weeks and stored in airtight plastic bags at 4°C. Analysis demonstrated that PEITC was stable in the diet under these conditions. The powdered diet was administered using metal boxfeeders (Lab Products Inc., Seafood, DE), which allow monitoring of food consumption and minimize diet waste. Fresh diet was provided every 3–4 days. Food consumption was monitored weekly when the mice were on the powdered diet. One week after treatment with the dietary compounds, carcinogen administration commenced. The mice in Groups 1–11 were treated by gavage with a mixture of B[a]P plus NNK (3 μmol each) in 0.1 ml cottonseed oil, once weekly for eight treatments. The mice in Group 12 were treated with cottonseed oil only. The mice were maintained on the AIN-93G powdered diet with or without the isothiocyanates or conjugates until one week following the final carcinogen administration. The diet was then changed to AIN-93M pelleted diet for all groups. The experiment was terminated 19 weeks after the final carcinogen administration. Lung tumors were scored as previously described [25].

The study design for Experiment 2 is summarized in Figure 2 and Table II. All groups contained 20 mice initially, except the group treated with B[a]P plus NNK and no dietary additions. This group had 30 mice. The mice were maintained on AIN-93G powdered diet, with or without chemopreventive agents, until 1 week following the end of carcinogen treatment as in Experiment 1. Then they diet, with or/and without chemopreventive agents. The chemopreventive agents were added to the diet at concentrations as follows: PEITC-NAC, 3 μmol/g diet (978 p.p.m.); MI, 55.5 μmol/g diet (10 000 p.p.m.); PEITC-NAC plus MI, 3 μmol/g diet plus 55.5 μmol/g diet. In Groups 2–4, the chemopreventive agents were added to the diet from one week prior to the first carcinogen treatment until one week after the last carcinogen treatment. B[a]P plus NNK were administered as in Experiment 1. In Groups 5–7, the mice were switched to diet containing the chemopreventive agents one week after completion of carcinogen administration. In Groups 8–10, the chemopreventive agents were added to the diet from one week prior to carcinogen treatment until the end of the experiment. In Groups 11–13, the chemopreventive agents were added to the diet beginning 24 h after the fourth of eight carcinogen treatments until the end of the experiment. In Groups 14–16, the chemopreventive agents were added to the diet beginning 24 h after the sixth of eight carcinogen treatments until the end of the experiment. New diet was provided every 3–4 days in all groups treated with chemopreventive agents. The experiment was terminated 19 weeks after the final carcinogen treatment and lung tumors were scored.

**Statistical analyses**

Repeated measures analysis of variance was used to determine the effects of time and treatment on the average body weight per mouse. Analysis of variance was also used to determine whether the number of lung tumors or their size differed among the treatments. Contrasts were constructed as necessary to examine comparisons of interest and tested using F-tests or t-tests. All analyses were adjusted for the number of comparisons made, using the method of Bonferroni.

**Results**

The purpose of Experiment 1 was to compare the efficacy of several isothiocyanates and their N-acetylcyesteine conjugates as inhibitors of lung tumor induction in the B[a]P plus NNK model. Earlier, we showed that dietary PEITC was a good inhibitor in this system, but the other compounds had not been tested [24]. The doses were chosen based on our previous work, in which we observed significant inhibition of lung tumor multiplicity by PEITC, either 3 or 1 μmol/g diet, administered during the carcinogen treatment phase [24].

There were no major effects of the isothiocyanates and their N-acetylcyesteine conjugates on body weights (Table I) or food consumption, which averaged 2.2 g/day. Effects on lung tumorigenecity are summarized in Table I. None of the compounds affected tumor incidence, which was 100% in all groups. PEITC significantly reduced tumor multiplicity by 22.8% and 19.4%, respectively, at the higher and lower doses. PEITC-NAC, PPITC, and PPITC-NAC all significantly reduced lung tumor multiplicity at the higher doses, by 38.2, 34.4 and 39.6%, respectively, but none of these compounds
Figure 1. Study design for effects of MI and PEITC-NAC individually and in combination, on lung tumor induction by B[a]P + NNK in A/J mice (Experiment 2).

was inhibitory at the lower doses. BITC-NAC did not inhibit tumor multiplicity at either dose. The 22.8% reduction in lung tumor multiplicity observed in mice treated with the higher dose of PEITC was lower than the 44% reduction observed previously at this dose, and we have no explanation for this discrepancy [24]. Jiao et al. previously showed that PEITC and PEITC-NAC had similar efficacy against lung tumor induction by NNK in A/J mice, and our results in the B[a]P plus NNK model indicate that inhibition by PEITC is due mainly to its effects on NNK [24,26,27]. Therefore, we expected that PEITC-NAC would reduce lung tumor multiplicity by ~40%, as we observed. Based on these results and the lower toxicity of PEITC-NAC than PEITC, we chose PEITC-NAC for Experiment 2.

The design of Experiment 2 is illustrated in Figure 2. Our goal was to investigate the efficacy of PEITC-NAC, MI and a mixture of these two agents at different stages of the tumor induction process. In this initial study, we restricted the design to a single dose of each agent or combination. We chose the 3 µmol/g diet dose for PEITC-NAC based on the results of Experiment 1, and the 55.5 µmol/g diet dose for MI based on the results of our recent dose–response study [15]. These doses were also used in the combination groups. There were five temporal sequences for administration of the chemopreventive agents (Figure 2 and Table II): during carcinogen treatment (Groups 2–4 of Table II), post-carcinogen treatment (Groups 5–7), administration during the entire experiment (Groups 8–10), administration starting halfway through the carcinogen treatment period and continuing for the duration of the experiment (Groups 11–13), and administration starting 75% through the carcinogen treatment period and continuing for the duration of the experiment (Groups 14–16).

There were minimal effects of the treatments on body weights as summarized in Table II and there were no effects on food consumption. There were no effects on lung tumor incidence. Effects on lung tumor multiplicity are summarized in Table II and Figure 3. PEITC-NAC significantly reduced lung tumor multiplicity by 31.5% when given during the carcinogen treatment phase. MI caused a marginally significant 25.7% reduction, while the combination reduced tumor multiplicity significantly by 41.4%. PEITC-NAC was ineffective when given in the post-carcinogen treatment phase. MI significantly reduced tumor multiplicity by 26.4%, while the combination of PEITC-NAC and MI significantly reduced tumor multiplicity by 40.2%. When administered throughout the experiment, all treatments reduced lung tumor multiplicity significantly, PEITC-NAC by 27.8%, MI by 51.0% and the combination by 64.7%. MI caused a marginally significant 25.7% reduction, while the combination reduced tumor multiplicity significantly by 41.4%. PEITC-NAC was ineffective when given in the post-carcinogen treatment phase. MI significantly reduced tumor multiplicity by 26.4%, while the combination of PEITC-NAC and MI significantly reduced tumor multiplicity by 40.2%. When administered throughout the experiment, all treatments reduced lung tumor multiplicity significantly, PEITC-NAC by 27.8%, MI by 51.0% and the combination by 64.7%. All treatments also significantly reduced tumor multiplicity when begun halfway through the carcinogen treatment period, PEITC-NAC by 28.6%, MI by 41.1% and the combination by 57.6%. However, when chemoprevention began 75% through the carcinogen administration phase, only MI and the combination of PEITC-NAC and MI significantly reduced lung tumor multiplicity, by 44.7 and 49.8%, respectively. PEITC-NAC was ineffective (5.5% reduction).

Comparisons of efficacy of different agents within each temporal sequence are summarized in Table III. There was no significant difference in efficacy among the agents given in the carcinogen administration phase. PEITC-NAC plus MI and MI were significantly more effective than PEITC-NAC when these agents were administered post-carcinogen only, throughout the entire experiment, or beginning 75% through the carcinogen treatment period. When administered beginning
Table II. Effects of PEITC-NAC and MI; individually and in combination, on lung tumor induction by B(a)P and NNK in A/J mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Compound</th>
<th>Carcinogen</th>
<th>No. of mice at termination</th>
<th>Mean body weight (g ± sd)</th>
<th>Lung tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>At termination</td>
<td>% reduction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% of mice with lung tumors</td>
<td>Tumors per mouse ± S.D.</td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>none</td>
<td>B[a]P + NNK</td>
<td>28</td>
<td>20.4 ± 1.1</td>
<td>24.2 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>carcinogen</td>
<td>PEITC-NAC</td>
<td>B[a]P + NNK</td>
<td>18</td>
<td>19.9 ± 1.2</td>
<td>23.3 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>treatment phase</td>
<td>MI</td>
<td>B[a]P + NNK</td>
<td>14</td>
<td>19.8 ± 1.8</td>
<td>23.5 ± 1.6</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>PEITC-NAC + MI</td>
<td>B[a]P + NNK</td>
<td>18</td>
<td>19.7 ± 1.3</td>
<td>23.3 ± 1.8</td>
</tr>
<tr>
<td>5</td>
<td>post-carcinogen</td>
<td>PEITC-NAC</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>19.6 ± 1.2</td>
<td>23.1 ± 1.0</td>
</tr>
<tr>
<td>6</td>
<td>treatment phase</td>
<td>MI</td>
<td>B[a]P + NNK</td>
<td>17</td>
<td>18.9 ± 0.6</td>
<td>23.0 ± 1.1</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>PEITC-NAC + MI</td>
<td>B[a]P + NNK</td>
<td>17</td>
<td>19.3 ± 1.6</td>
<td>23.2 ± 1.6</td>
</tr>
<tr>
<td>8</td>
<td>entire experiment</td>
<td>PEITC-NAC</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>20.2 ± 0.6</td>
<td>23.2 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>MI</td>
<td>B[a]P + NNK</td>
<td>18</td>
<td>19.8 ± 1.3</td>
<td>22.7 ± 2.4</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>PEITC-NAC + MI</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>20.0 ± 1.0</td>
<td>22.9 ± 1.0</td>
</tr>
<tr>
<td>11</td>
<td>50% of carcinogen</td>
<td>PEITC-NAC</td>
<td>B[a]P + NNK</td>
<td>20</td>
<td>20.1 ± 1.3</td>
<td>22.9 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>treatment phase</td>
<td>MI</td>
<td>B[a]P + NNK</td>
<td>18</td>
<td>19.4 ± 1.7</td>
<td>22.3 ± 3.1</td>
</tr>
<tr>
<td>13</td>
<td>until termination</td>
<td>PEITC-NAC + MI</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>20.3 ± 1.7</td>
<td>23.6 ± 1.9</td>
</tr>
<tr>
<td>14</td>
<td>75% of carcinogen</td>
<td>PEITC-NAC</td>
<td>B[a]P + NNK</td>
<td>18</td>
<td>19.5 ± 1.5</td>
<td>22.6 ± 1.8</td>
</tr>
<tr>
<td>15</td>
<td>treatment phase</td>
<td>MI</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>19.6 ± 1.0</td>
<td>22.9 ± 2.2</td>
</tr>
<tr>
<td>16</td>
<td>until termination</td>
<td>PEITC-NAC + MI</td>
<td>B[a]P + NNK</td>
<td>19</td>
<td>20.0 ± 1.0</td>
<td>22.8 ± 0.8</td>
</tr>
<tr>
<td>17</td>
<td>–</td>
<td>none</td>
<td>none</td>
<td>19</td>
<td>20.2 ± 1.8</td>
<td>24.2 ± 2.1</td>
</tr>
</tbody>
</table>

*Beginning at age 7–8 weeks, groups of 20 female A/J mice (except Group 1, 30 mice) were treated by gavage weekly for 8 weeks with a mixture of B[a]P + NNK (3 μmol each) in 0.1 ml cottonseed oil. The mice were maintained on AIN-93G diet from age 5–6 weeks until 1 week after the end of carcinogen treatment, then shifted to AIN-93G diet for the duration of the experiment. Chemopreventive agents were added to the diet according to the temporal sequences shown in the Table. The mice were killed 19 weeks after the final carcinogen treatment.

*Compared with Group 1.
Inhibition of lung tumorigenesis

Fig. 3. Effects of PEITC-NAC, MI and PEITC-NAC plus MI on lung tumorigenesis by B[α]P plus NNK in A/J mice in different temporal sequences. *P < 0.05.

Table III. Summary of comparisons among different agents in each temporal sequence

<table>
<thead>
<tr>
<th>Temporal sequence of chemopreventive agents</th>
<th>Significant differences among agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinogen treatment phase</td>
<td>None</td>
</tr>
<tr>
<td>Post-carcinogen treatment phase</td>
<td>PEITC-NAC + MI and MI &gt; PEITC-NAC</td>
</tr>
<tr>
<td>Entire experiment</td>
<td>PEITC-NAC + MI and MI &gt; PEITC-NAC</td>
</tr>
<tr>
<td>50% of carcinogen treatment phase until termination</td>
<td>PEITC-NAC + MI &gt; PEITC-NAC</td>
</tr>
<tr>
<td>75% of carcinogen treatment phase until termination</td>
<td>PEITC-NAC + MI and MI &gt; PEITC-NAC</td>
</tr>
</tbody>
</table>

50% through the carcinogen treatment phase, PEITC-NAC plus MI was significantly more effective than PEITC-NAC. There were no significant differences among the other agents in this temporal sequence.

Comparative efficacy was also analyzed for all groups combined treated with a given agent, independent of the temporal sequence. The combination of PEITC-NAC plus MI was significantly more effective than MI alone (P = 0.0015) or PEITC-NAC alone (P < 0.0001). MI was significantly more effective than PEITC-NAC (P < 0.0001).

The effects of duration of treatment for all groups treated with chemopreventive agents after carcinogen administration (e.g. Groups 5–16) were analyzed. The results are presented in Figure 4. There was a significant trend for increased reduction in lung tumor multiplicity with increased duration of treatment for PEITC-NAC (P = 0.0007), MI (P = 0.015), and PEITC-NAC plus MI (P = 0.0045).

Stomach tumors were observed in all groups treated with B[α]P plus NNK in both experiments. In our hands, these tumors are difficult to quantify because they congeal into a mass. We did not detect major effects of the chemopreventive agents on these tumors.

Discussion

Our results provide some encouraging data pertinent to the two major questions posed in this study. First, a mixture of chemopreventive agents (PEITC-NAC plus MI) was effective as an inhibitor of lung tumor multiplicity in mice treated with B[α]P plus NNK. The combination of PEITC-NAC plus MI was more effective than the agents given alone in each temporal sequence, and, when all the data were combined, the combination was significantly more effective than either agent alone. Second, with one exception, significant inhibition of lung tumorigenicity was observed when administration of the agents was begun 50 or 75% into the carcinogen administration phase. This is apparently the first study to demonstrate inhibition in these temporal sequences. The results of this initial study of mixtures and temporal sequences provide a platform on which further to develop realistic approaches to chemoprevention of smoking induced lung cancer. The use of multiple chemopreventive agents is a potentially practical way to combat the multiple aberrations leading to lung cancer.

There was consistency in the results presented here. First, inhibition of lung tumor multiplicity by PEITC-NAC, given during the carcinogen administration phase, was similar in Experiments 1 (38.2%) and 2 (31.5%). Second, the lack of efficacy of PEITC-NAC, given in the post-carcinogen administration phase, is consistent with earlier results obtained with the same dose of dietary PEITC in mice treated with NNK only [28]. However, Chung et al. have observed that higher doses of PEITC-NAC and BITC-NAC (15 µmol/g diet) inhibit lung tumorigenicity when given after administration of B[α]P [29]. Third, the results obtained with 1% MI are similar to those described previously in that significant inhibition was observed in both the carcinogen administration phase and in the post-carcinogen phase, although the extent of inhibition
was somewhat less than in our previous study [15]. Fourth, there was a significant positive relationship between duration of treatment and extent of inhibition for all the treatments that extended into the post-carcinogen treatment phase. Finally, the mixture of PEITC-NAC and MI was consistently superior to the treatments alone, although these differences were not significant in any of the individual temporal sequences.

Further studies are required to evaluate more fully the efficacy of PEITC-NAC and the mixture of PEITC-NAC and MI. Only one dose of PEITC-NAC, 3 µmol/g diet, was tested here. Doses of PEITC-NAC as high as 15 µmol/g diet have been used before, with marginal toxicity [29]. It is likely that doses of PEITC-NAC higher than 3 µmol/g diet, alone and in combination with MI, would inhibit more effectively than observed here. Further evaluation of the potential additive or synergistic effects of different doses of PEITC-NAC and MI is also required. A second parameter that deserves further consideration is the carcinogen dose. The dose used here, 3 µmol of each carcinogen once weekly for 8 weeks, is equivalent to ~69 mg/kg carcinogen at each dose. This is nearly 40 times higher than each single dose of NNK used to induce lung tumors in rats and far higher than the dose to a smoker [30]. Lower individual doses of 2 and 1 µmol of each carcinogen used in this model produce 7.3 and 3.3 lung tumors per mouse, respectively [19]. Third, duration of treatment with chemopreventive agents could be extended. The data presented in Figure 4 suggest that improved efficacy could be obtained with longer treatment periods after carcinogen administration.

This study directly compares the efficacy of an isothiocyanate derivative (PEITC-NAC) with MI. Previous data clearly demonstrate that PEITC is more effective than MI as an inhibitor of mouse lung tumorigenesis, per mol of inhibitor, when given during the carcinogen administration phase [15,24]. However, MI is considerably less toxic than PEITC and PEITC-NAC, allowing use of far higher doses. In this study, PEITC-NAC and MI had similar efficacy when given during the carcinogen administration phase. MI was significantly more effective than PEITC-NAC in all the other temporal sequences except when the agents were started 50% through the carcinogen administration phase. In that case, MI was also more effective than PEITC-NAC, but the difference was not significant. These results indicate that MI is a more effective chemopreventive agent against mouse lung tumorigenesis than PEITC-NAC. PEITC, however, is a highly effective inhibitor of NNK induced lung tumorigenesis in the F-344 rat [30–32]; no data are available for MI in that model.

Isothiocyanates and their N-acetylcysteine conjugates are known to affect carcinogen metabolizing enzymes in a favorable manner [16,33]. Abundant evidence demonstrates that these compounds are selective inhibitors of cytochrome P450 enzymes involved in the metabolic activation of carcinogens. They are also inducers of phase 2 enzymes involved in carcinogen detoxification. Dietary PEITC, given during the carcinogen administration period, inhibits lung tumorigenesis by B[a]P plus NNK partly by decreasing DNA pyridyloxobutylation by NNK [27]. PEITC-NAC acts in part by dissociation to PEITC, therefore one would expect a similar mechanistic outcome [34]. Recent studies demonstrate that isothiocyanates and their N-acetylcysteine conjugates induce apoptosis, which is also likely to play a significant role in their chemopreventive properties [29,35–40]. Much less is known about mechanisms of chemoprevention by MI. No data are available with respect to MI effects on carcinogen metabolism or apoptosis. However, MI is known to reverse the dedifferentiating effect of B[a]P-7,8-diol-9,10-epoxide in human lung cells, which may relate to its chemopreventive effects [41]. In the study reported here, PEITC-NAC significantly inhibited lung tumor multiplicity in the carcinogen treatment phase while MI significantly inhibited in the post-carcinogen treatment phase. These results indicate that PEITC-NAC and MI have different, and perhaps complementary, mechanisms of action. This could be a key factor influencing the efficacy of a mixture of these agents.

In conclusion, this study provides useful new data relevant to some important issues in chemoprevention of lung cancer. The results demonstrate combined efficacy of PEITC-NAC and MI, which could potentially be improved with different dose combinations or duration of treatment. The results also show that treatment with chemopreventive agents can begin at a late stage of carcinogen exposure and still be effective. This point is important when considering chemoprevention of smoking-related cancer.

Acknowledgements

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References


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