Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in N-nitrosobis(2-oxopropyl)amine-treated hamsters

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The chemopreventive effects of phenethyl isothiocyanate (PEITC) were investigated in N-nitrosobis(2-oxopropyl)amine (BOP)-treated hamsters. Female 5-week-old Syrian golden hamsters were divided into six groups. Animals in groups 1-3, each consisting of 30 hamsters, were given BOP by two subcutaneous injections 7 days apart at a dose of 20 mg/kg body weight, plus either 100, 10 or 0 µmol of PEITC in corn oil by gavage 2 h prior to each BOP treatment, respectively per group. Animals in groups 4 and 5, each consisting of 10 hamsters, were given 100 and 10 µmol of PEITC alone in corn oil, and 10 animals in group 6 served as a vehicle control. Animals were sacrificed 52 weeks after the first BOP injection. Both the incidences and multiplicities of lung adenomas and/or adenocarcinomas were significantly decreased in a dose-dependent manner by PEITC treatments (P < 0.01 or 0.05). The lung tumor incidences were inhibited by 100% with 100 µmol PEITC and by 82% with the 10 µmol dosage. In addition, the high dose of PEITC also significantly inhibited pancreatic carcinogenesis (P < 0.05) and showed a tendency to lower the incidences of liver and renal tumors, although these effects were not statistically significant. Under the present experimental conditions, PEITC itself did not cause any apparent toxicity. Our results thus indicate that PEITC is a remarkably effective chemopreventive agent for the BOP-induced lung and pancreatic tumors in hamsters.

Phenethyl isothiocyanate (PEITC*), a natural constituent of cruciferous vegetables, has been extensively investigated for its chemopreventive activity against cancer in rats and mice but not in hamsters (1-8). In rats and mice, PEITC effectively inhibited chemically-induced lung, mammary gland, forestomach and esophagus tumorigenesis (8). Principal mechanisms underlying the chemopreventive effects of PEITC are related to its ability to attenuate DNA alkylaition levels induced by chemical carcinogens such as 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (1,2,9), N-nitrosobenzylmethylamine (BOP) (10,11), (purity >99%) was purchased from Aldrich Chemical Co. 2,6-Dimethylnitrosamine (DMN) (20,21) was purchased from Nacalai Tesque (Kyoto, Japan). PEITC (purity >99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Animals in groups 1-3, each consisting of 30 hamsters, were twice given 7 days apart, subcutaneous injections of BOP at a dose of 20 mg/kg body weight or 127 µmol/kg body weight. At 2 h prior to each BOP treatment, the animals in groups 1 and 2 were twice given by gavage either 100 µmol or 10 µmol PEITC in 0.1 ml corn oil, respectively. Hamsters in group 3 were treated with BOP alone, serving as a positive control. Groups 4, 5 and 6, each consisting of 10 hamsters, received 100 µmol PEITC, 10 µmol PEITC and corn oil alone, respectively (Figure 1). The doses of PEITC used in the present study were determined according to previous experiments in rats and mice (2-5,7). The hamsters were observed daily and weighed once every 4 weeks. At the end of week 52, all surviving animals were killed and examined. Murbud or dead animals were also completely autopsied for histological examination. At autopsy, the main target organs of BOP such as pancreas, lung, liver and kidney were examined macroscopically and then fixed in 10% phosphate buffered formalin. These organs were processed for histological examination by conventional methods, and sections stained with hematoxylin and eosin. All proliferative lesions were diagnosed histopathologically and counted in representative sections. The tumor incidences were analyzed by the Fisher's exact probability test and the tumor multiplicities were evaluated by the analysis of variance with Dunnet’s t-test.

*Abbreviations: PEITC, phenethyl isothiocyanate; BOP, N-nitrosobis(2-oxopropyl)amine; NNK, 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone.

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were observed in the BOP-treated hamsters after receiving dose-dependent manner by PEITC. In fact, no lung tumors previously (16). As shown in Table I, the incidence of total lung tumors in groups 1 and 2 was significantly inhibited in a dose-dependent manner by PEITC. The multiplicity of adenomas and adenocarcinomas) was reduced by 100% (P < 0.01) and 82% (P < 0.01) by PEITC at doses of 100 ¡ìmol and 10 ¡ìmol/hamster. The multiplicity data were more striking: the PEITC treatments decreased the multiplicity of adenocarcinomas as well as adenomas in a clear dose-dependent manner. These results show that PEITC exerted a remarkable inhibitory activity against BOP-induced lung tumorigenesis in hamsters.

Cancerous and precancerous ductal lesions observed in the exocrine pancreas consist of adenocarcinomas and dysplasias as reported previously (16). Four anatomical parts of the pancreas (gastric, splenic and duodenal lobes, and head portion) were sectioned in each animal, for investigation of the multiplicity of dysplasias, as is usual in our laboratory (16–18). Incidences and multiplicities for these pancreatic proliferative lesions in all the groups are summarized in Table II. The incidence of dysplastic lesions was significantly reduced from 82% (P < 0.01) by PEITC at doses of 100 ¡ìmol and 10 ¡ìmol/hamster. The multiplicity of pancreatic lesions (dysplasias and adenocarcinomas) was also greatly lowered in the group treated with high dose of PEITC at 100 ¡ìmol dose (P < 0.05). In addition, the incidence of total pancreatic proliferative lesions was significantly decreased in group 1 as compared to group 3 (P < 0.05). The multiplicity of pancreatic lesions (dysplasias and adenocarcinomas) was also greatly lowered in the group treated with high dose of PEITC as compared to groups 2 (P < 0.05) and 3 (P < 0.01). Liver tumors found in the BOP-treated animals were histologically classified as hepatocellular and cholangiocellular adenomas. The incidences of these liver tumors showed a dose-dependent decrease with PEITC, although these decreases were not statistically significant. Kidney tumors were diagnosed as renal cell adenomas and nephroblastomas. The incidences of kidney tumors were decreased by the PEITC treatments, but again were not statistically significant. Histopathological examination showed that no neoplastic lesions were present in lung, pancreas and kidney of animals treated with PEITC alone (groups 4 and 5).

Table I. Incidence and multiplicity of pancreatic neoplastic and preneoplastic lesions in hamsters treated with PEITC and/or BOP

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no. of animals</th>
<th>Incidence (%)</th>
<th>Multiplicity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AD⁴</td>
<td>ADC</td>
</tr>
<tr>
<td>1. 100 ¡ìmol PEITC + BOP</td>
<td>29</td>
<td>0**</td>
<td>0*</td>
</tr>
<tr>
<td>2. 10 ¡ìmol PEITC + BOP</td>
<td>29</td>
<td>3 (10.3)**</td>
<td>1 (3.4)</td>
</tr>
<tr>
<td>3. BOP alone</td>
<td>30</td>
<td>16 (53.3)</td>
<td>7 (23.3)</td>
</tr>
<tr>
<td>4. 100 ¡ìmol PEITC alone</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5. 10 ¡ìmol PEITC alone</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6. Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

⁴AD, adenoma; ADC, adenocarcinoma.
Significantly different from BOP alone group at *P < 0.05 and **P < 0.01.

Table II. Incidence and multiplicity of pancreatic neoplastic and preneoplastic lesions in hamsters treated with PEITC and/or BOP

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective no. of animals</th>
<th>Incidence (%)</th>
<th>Multiplicity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ADC³</td>
<td>DYS</td>
</tr>
<tr>
<td>1. 100 ¡ìmol PEITC + BOP</td>
<td>29</td>
<td>5 (17.2)</td>
<td>4 (13.8)*</td>
</tr>
<tr>
<td>2. 10 ¡ìmol PEITC + BOP</td>
<td>29</td>
<td>11 (37.9)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>3. BOP alone</td>
<td>30</td>
<td>12 (40.0)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>4. 100 ¡ìmol PEITC alone</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5. 10 ¡ìmol PEITC alone</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6. Control</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

³ADC, adenocarcinoma; DYS, dysplastic lesion.
Significantly different from BOP alone group at *P < 0.05 and **P < 0.01.
Significantly different from 10 ¡ìmol PEITC + BOP group at ***P < 0.05.

Fig. 1. Experimental protocol.

No significant differences in mean body weight gain were found in the groups, and the final body weights were not statistically different between groups. No significant differences in absolute organ weights were noted between groups. No differences in relative organ weights were found between groups except for the kidneys which were slightly but significantly increased in group 1 as compared to the value for group 2 (P < 0.05). Lung tumors in BOP-treated hamsters were diagnosed as adenomas and adenocarcinomas as described previously (16). As shown in Table I, the incidence of total lung tumors in groups 1 and 2 was significantly inhibited in a dose-dependent manner by PEITC. In fact, no lung tumors were observed in the BOP-treated hamsters after receiving 100 ¡ìmol of PEITC. The formation of lung tumors (adenomas and adenocarcinomas) was reduced by 100% (P < 0.01) and 82% (P < 0.01) by PEITC at doses of 100 ¡ìmol and 10 ¡ìmol/hamster. The multiplicity data were more striking: the PEITC treatments decreased the multiplicity of adenocarcinomas as well as adenomas in a clear dose-dependent manner. These results show that PEITC exerted a remarkable inhibitory activity against BOP-induced lung tumorigenesis in hamsters.
In addition, no obvious toxicity was evident in the organs of hamsters treated with PEITC alone.

Results of the present study show that PEITC is remarkably efficacious in inhibiting lung tumorigenesis in hamsters treated with BOP. This study is the first to show the chemopreventive activity of PEITC in hamsters. The inhibitory effects found in the present study were quite dramatic, since a total blockage of lung tumor formation was accomplished at a 100 μmol dose of PEITC. This study also shows for the first time a significant inhibitory effect of PEITC against pancreatic carcinogenesis, although a previous bioassay in rats with NNK has shown a possibly enhancing activity of PEITC in pancreatic carcinogenesis (1).

The underlying mechanism of tumor inhibition by PEITC against hamster lung or pancreatic tumorigenesis remains to be elucidated. However, a mechanism similar to that observed in rats and mice could be involved, namely, arylalkyl isothiocyanates acting as anti-initiating or blocking agents (3,12). This is supported by the fact that the inhibition was achieved by administering PEITC only 2 h prior to carcinogen treatment. Previous studies in rats and mice have shown that the inhibitory effects of PEITC on NNK lung carcinogenesis are due to its inhibition of P450-mediated metabolic α-hydroxylation to the alkylation agents (1,2,4,11-13). It has also been reported that PEITC and related isothiocyanates inhibit hamster microsomal metabolism of NNK (22,23).

BOP, a known pancreas-specific nitrosamine in hamsters, causes DNA methylation primarily, although low levels of hydroxypropylation could also occur, in the target organs including the pancreas and lung (24). It has been shown that BOP is activated by CYP2B1 and other related forms in mice, rats and hamsters (25). CYP2B1 and CYP2E1 have been shown to activate NNK in rat or mouse lung microsomes (13,26). The involvement of CYP2B1 as well as CYP2E1 in NNK metabolism in hamsters has also been suggested by immunoblot analyses (27). It is therefore possible that BOP and NNK may share the major P450 forms for their activation in hamsters. It has been reported that PEITC is rapidly distributed in the liver, lung and other tissues of mice (28). Conceivably this could occur in hamsters, the inhibition of BOP-induced DNA methylation by PEITC may be at least partially attributable to the reduction of lung tumor development.

Isothiocyanates are produced in plants when myrosinase is released as a result of plant rupture. Humans are exposed to PEITC through consumption of cruciferous vegetables, for example, watercress is a rich source of PEITC (29). PEITC used in this study was not toxic at the doses tested. Compounds which strongly inhibit carcinogenesis at doses that do not alter normal physiological or detoxification functions are candidates for chemopreventive studies in humans. In this context, PEITC appears to fall into this category. Furthermore, human intake of dietary PEITC could contribute to primary prevention of cancer. In addition to the ability to suppress metabolic activation of carcinogenic nitrosamines, a number of dietary isothiocyanates are also known to induce phase II enzymes such as glutathione S-transferase and quinone oxidoreductase (30,31). This dual protective mechanism makes PEITC a potential agent against a wide spectrum of toxic or carcinogenic compounds.

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References
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