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# Chemopreventive Effects of 3-Phenylpropyl Isothiocyanate on Hamster Lung Tumorigenesis Initiated with *N*-Nitrosobis(2-oxopropyl)amine

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**The chemopreventive effects of 3-phenylpropyl isothiocyanate (PPITC) were investigated in *N*-nitrosobis(2-oxopropyl)amine (BOP)-initiated hamsters. A total of 120 female 5-week-old hamsters were divided into 6 groups. Animals in groups 1–3, each consisting of 30 hamsters, were twice *sc* injected 7 days apart as an initiation treatment. Hamsters in groups 1 and 2 were respectively given 100 μmol and 10 μmol of PPITC by gavage 2 h prior to each BOP treatment. Animals in group 3 were treated with BOP alone, serving as an initiation-positive control. Animals in groups 4–6, each consisting of 10 hamsters, were given 100 μmol or 10 μmol of PPITC alone, or non-treated, thus being available as matched negative controls to groups 1–3. At termination (experimental week 51 after the first BOP injection), the incidences of lung adenomas and/or adenocarcinomas were significantly decreased in groups 1 and 2 as compared to the group 3 value ($P < 0.01$). The combined lung tumor incidences were inhibited by 94% and 59% at 100 and 10 μmol doses, respectively. The inhibitory effects of PPITC were thus dose-dependent. The data for multiplicity of lung tumors dramatically illustrated the inhibitory effects of PPITC, and there were also statistically significant differences in the chemopreventive effect between 100 μmol and 10 μmol PPITC treatments. On the other hand, the PPITC treatments did not significantly modulate the development of neoplastic lesions in the pancreas, liver and kidney, although the treatments did show inhibitory tendencies, except on the liver lesions. Under the present experimental conditions, PPITC itself did not exhibit tumorigenicity or apparent toxicity. The results in the present study thus clearly indicate that PPITC has an effective chemopreventive action on BOP-induced lung tumorigenesis in hamsters.**

Key words: PPITC — Chemoprevention — Lung — BOP — Hamster

Allyl isothiocyanates occur naturally in many of the vegetables, food additives and flavoring agents consumed by humans.[1] Phenethyl isothiocyanate (PEITC), a constituent of cruciferous vegetables, has been extensively investigated for chemopreventive action in rodent chemical carcinogenesis models.[2–8] In rat and mouse experiments, PEITC effectively inhibited chemically induced lung,[2–6] mammary gland,[7] forestomach[7] and esophagus[8,9] tumorigenesis. It has been shown in studies on the structure-activity relationships of isothiocyanates that synthetic analogues of PEITC with longer alkyl chains have a higher ability to reduce DNA alkylation than PEITC.[3,5] 3-Phenylpropyl isothiocyanate (PPITC), a longer alkyl chain homologue of PEITC, has greater chemopreventive effects on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in mice than does PEITC.[4–6]

*N*-Nitrosobis(2-oxopropyl)amine (BOP) has been shown to induce lung, pancreatic, liver and kidney tumors in Syrian golden hamsters[10] and the BOP-pancreatic carcinogenesis model in this animal species has been extensively studied because of the histological and biological similarities between human and hamster pancreas tumors.[10] The hamster is also well documented as suitable for examining the effects of carcinogenic substances which may target the respiratory tract.[11] In the present study, the effects of PPITC were investigated in BOP-initiated hamsters.

## MATERIALS AND METHODS

**Animals and chemicals** A total of 120 female Syrian golden hamsters (Japan SLC, Inc., Shizuoka), 5 weeks old and weighing approximately 80 g at the commencement, were used in this experiment. The animals were housed, five per plastic cage, in an air-conditioned room at 23±2°C and 60±5% humidity under a daily cycle of alternating 12-h periods of light and darkness. Oriental M (Oriental Yeast Co., Ltd., Tokyo) was used as the basal diet. BOP was obtained from Nacalai Tesque (Kyoto) and PPITC (purity >98%) from Lancaster Chemical Co. (Morecambe, U.K.).

**Experimental design** Animals in groups 1–3, each consisting of 30 animals, were twice (7 days apart) given subcutaneous injections of BOP at a dose of 20 mg/kg body weight as the initiation treatment. Two hours prior

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to each BOP administration, the animals in groups 1 and 2 were given by gavage 100 $\mu$mol of PPITC (approximately 220 mg/kg) and 10 $\mu$mol of PPITC (approximately 22 mg/kg) dissolved in 0.1 ml of corn oil, respectively. Hamsters in group 3 were treated with BOP alone, serving as an initiation-positive control. Groups 4, 5 and 6, each consisting of 10 animals, twice received 100 $\mu$mol of PPITC, 10 $\mu$mol of PPITC or corn oil alone, respectively, thus serving as initiation-negative matched controls to groups 1, 2 and 3 (Fig. 1). The doses of PPITC used in the present study were determined based on the previous experiments in mice.[4-6]

The hamsters were observed daily and weighed once every 4 weeks. At the end of week 51, all surviving animals were killed and examined. Moribund or dead animals were also completely autopsied for histological examination. At autopsy, the main target organs for BOP-tumorigenicity, such as the pancreas, lung, liver and kidney, were carefully examined macroscopically, and then fixed in 10% phosphate-buffered formalin. These organs were processed for histological observation by conventional methods, and sections were stained with hematoxylin and eosin. All proliferative lesions were diagnosed histopathologically and counted in representative sections.

The results were statistically analyzed by means of Student's $t$-test and Fisher's exact probability test.

## RESULTS

No significant differences in mean body weight gain were found between groups (Fig. 2), and thus the final body weights were not statistically different between groups. As shown in Table I, no significant differences in absolute organ weights except the right kidney were noted between groups. Except for the kidneys, which were slightly but significantly decreased in weight in group 1 as compared to the group 3 value ($P<0.05$), no differences in relative organ weights were found between groups.

Histopathologically, lung tumors were classified into adenomas and adenocarcinomas. As shown in Table II, the incidences of adenomas in groups 1, 2 and 3 were respectively 3.4%, 23.3% and 53.6%, those of adenocarcinomas were 0%, 0%, 21.4% and those of combined lung tumors (adenomas and adenocarcinomas) were 3.4%, 23.3% and 57.1%. Thus, the developments of adenomas, adenocarcinomas and combined lung tumors were suppressed by 94%, 100% and 94% at 100 $\mu$mol dose, and by 57%, 100% and 59% at 10 $\mu$mol dose. The data for multiplicities of lung tumors showed greater dose-dependent inhibitory effects than those for incidences, and, in addition, there were significant differences in multiplicity of adenomas or combined lung tumors between groups 1 and 2 (Table II).

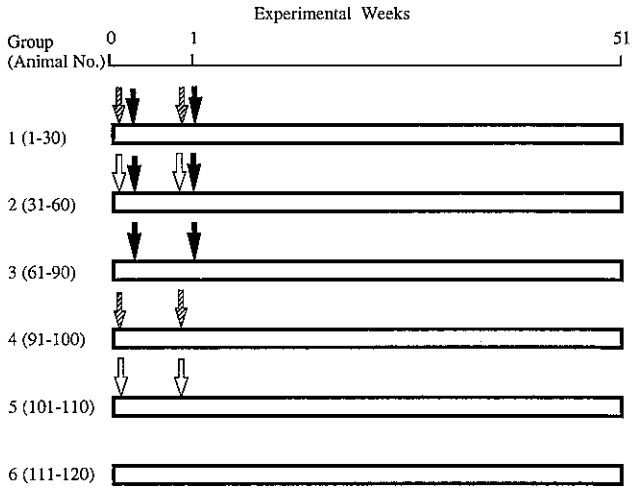


Fig. 1. Experimental design. $N$-Nitrosobis(2-oxopropyl)-amine (BOP, ⇓) was subcutaneously administered twice with an interval of a week, at a dose of 20 mg/kg. High (100 $\mu$mol/hamster, ⇓) and low (10 $\mu$mol/hamster, ⇩) doses of 3-phenylpropyl isothiocyanate (PPITC) in 0.1 ml of corn oil were given by gavage 2 h prior to each BOP treatment.

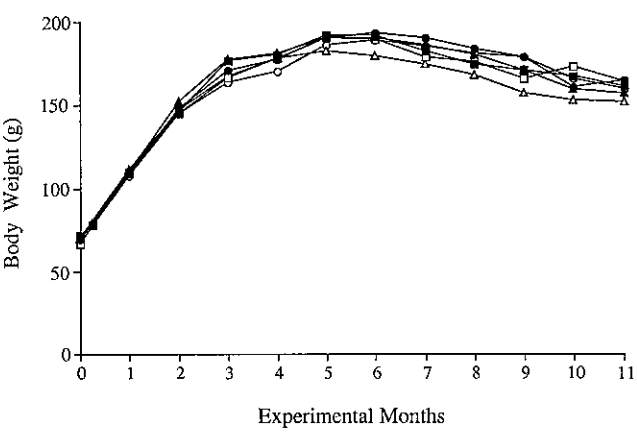


Fig. 2. Body weight curves for hamsters treated with PPITC and/or BOP. Symbols: ■, 100 $\mu$mol PPITC+BOP (group 1); ▲, 10 $\mu$mol PPITC+BOP (group 2); ●, BOP alone (group 3); □, 100 $\mu$mol PPITC alone (group 4); △, 10 $\mu$mol PPITC alone (group 5); ○, control (group 6).

Cancerous and precancerous ductal lesions observed in the exocrine pancreas were histologically classified as adenocarcinomas and dysplasias as reported previously.[12] Four anatomical parts of the pancreas (gastric, splenic and duodenal lobes, and head portion) were sectioned in each animal to investigate the multiplicity of dysplasias, as usual in our laboratory.[12] These pancreatic

Table I. Absolute and Relative Organ Weights of Hamsters Treated with PPITC and/or BOP

| Group | Lung (mg) | | | Liver (g) | Kidney (mg) | | |
|---|---|---|---|---|---|---|---|
| | R | L | R+L | | R | L | R+L |
| 1. 100 μmol PPITC+BOP | 658±87 (425) | 342±43 (221) | 1001±128 (647) | 8.80±4.00 (5.63) | 592±73* (383*) | 615±75 (398) | 1208±145 (781*) |
| 2. 10 μmol PPITC+BOP | 656±94 (450) | 338±51 (232) | 994±141 (682) | 7.94±2.15 (5.38) | 619±90 (424) | 649±128 (445) | 1268±196 (869) |
| 3. BOP alone | 717±130 (455) | 368±93 (232) | 1085±212 (687) | 8.59±2.56 (5.32) | 646±90 (407) | 656±88 (413) | 1301±173 (820) |
| 4. 100 μmol PPITC alone | 659±91 (420) | 337±34 (216) | 996±122 (636) | 6.44±1.54 (4.05) | 602±100 (381) | 635±107 (402) | 1237±201 (783) |
| 5. 10 μmol PPITC alone | 666±85 (448) | 348±54 (234) | 1014±136 (682) | 6.07±0.96 (4.06) | 579±55 (390) | 592±47 (399) | 1171±100 (789) |
| 6. Control | 644±72 (427) | 352±40 (235) | 996±108 (662) | 6.39±1.32 (4.20) | 597±128 (393) | 602±112 (397) | 1199±239 (790) |

Data represent mean values±SD.
Numbers in parentheses, relative organ weights.
*: Significantly different from the BOP-alone group at $P<0.05$.

Table II. Incidence and Multiplicity of Lung Tumors in Hamsters Treated with PPITC and/or BOP

| Group | Effective No. of animals | Incidence (%) | | | Multiplicity (Mean±SD) | | |
|---|---|---|---|---|---|---|---|
| | | AD[a)] | ADC[a)] | Total | AD | ADC | Total |
| 1. 100 μmol PPITC+BOP | 29 | 1 (3.4)** | 0* | 1 (3.4)** | 0.03±0.19**,*** | 0* | 0.03±0.19**,*** |
| 2. 10 μmol PPITC+BOP | 30 | 7 (23.3)* | 0* | 7 (23.3)** | 0.23±0.43** | 0* | 0.23±0.43** |
| 3. BOP alone | 28 | 15 (53.6) | 6 (21.4) | 16 (57.1) | 0.93±1.12 | 0.21±0.42 | 1.14±1.27 |
| 4. 100 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. 10 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. Control | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

a) AD, adenoma; ADC, adenocarcinoma.
*, **: Significantly different from the BOP-alone group at * $P<0.05$ and ** $P<0.01$.
***: Significantly different from the 10 μmol PPITC+BOP group at $P<0.05$.

Table III. Incidence and Multiplicity of Pancreatic Neoplastic and Preneoplastic Lesions in Hamsters Treated with PPITC and/or BOP

| Group | Effective No. of animals | Incidence (%) | | | Multiplicity (Mean±SD) | | |
|---|---|---|---|---|---|---|---|
| | | ADC[a)] | DYS[a)] | Total | ADC | DYS | Total |
| 1. 100 μmol PPITC+BOP | 29 | 14 (48.3) | 11 (37.9) | 20 (68.9) | 0.55±0.63 | 0.41±0.56 | 0.96±0.82 |
| 2. 10 μmol PPITC+BOP | 30 | 19 (63.3) | 12 (40.0) | 24 (80.0) | 0.83±0.79 | 0.60±0.81 | 1.43±1.16 |
| 3. BOP alone | 28 | 15 (53.5) | 11 (39.2) | 20 (71.4) | 0.85±1.04 | 0.67±1.05 | 1.53±1.45 |
| 4. 100 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. 10 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. Control | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

a) ADC, adenocarcinoma; DYS, dysplastic lesion.

neoplastic lesions were only noted in the BOP-treated groups. Incidence and multiplicity data for these lesions are summarized in Table III. Although the incidences of dysplastic lesions and adenocarcinomas were not statistically different between the BOP-treated groups, the multiplicity data for dysplastic lesions, adenocarcinomas or combined proliferative pancreatic lesions indicated that PPITC tends to exert dose-dependent inhibitory effects.

Liver tumors were histologically interpreted as hepatocellular and cholangiocellular adenomas, which were in-

Table IV. Incidence of Liver and Kidney Tumors in Hamsters Treated with PPITC and/or BOP

| Group | Effective No. of animals | Hepatocellular (%) | | | Cholangiocellular (%) | | | Kidney (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AD[a] | CA[a] | Total | AD | CA | Total | AD | NB[a] | Total |
| 1. 100 μmol PPITC+BOP | 29 | 9 (31.0) | 2 (6.9) | 10 (34.5) | 6 (20.7) | 5 (17.2) | 11 (37.9) | 0 | 0 | 0 |
| 2. 10 μmol PPITC+BOP | 30 | 5 (16.7) | 1 (3.3) | 5 (16.7) | 2 (6.7) | 7 (23.3) | 9 (30.0) | 1 (3.3) | 1 (3.3) | 2 (6.7) |
| 3. BOP alone | 28 | 9 (32.1) | 0 | 9 (32.1) | 2 (7.1) | 8 (28.6) | 10 (35.7) | 0 | 2 (7.1) | 2 (7.1) |
| 4. 100 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. 10 μmol PPITC alone | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. Control | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*a*) AD, adenoma; CA, carcinoma; NB, nephroblastoma.

frequently noted in the BOP-treated groups. The incidences of these liver tumors were not statistically significantly different between groups (Table IV). Kidney tumors were histologically diagnosed as adenomas and nephroblastomas. The incidence of nephroblastomas was dose-dependently reduced by the PPITC treatments, again without statistical significance (Table IV).

As shown in Tables II–IV, no neoplastic lesions were histopathologically observed in the lung, pancreas, liver and kidney of animals treated with PPITC alone (groups 4 and 5), as well as in control animals (group 6). In addition, no obvious toxic changes were found in these organs.

## DISCUSSION

The results in the present study clearly indicate that PPITC, an isothiocyanate analogous to PEITC, has a dose-dependent chemopreventive action against lung carcinogenesis in hamsters initiated with BOP. The chemopreventive action of PPITC against pancreatic carcinogenesis was weak, however. The underlying mechanism of the chemopreventive effects of PEITC against NNK-induced lung carcinogenesis in mice and rats has been suggested to be inhibition of P450s, as well as induction of phase II enzymes.[13–16] Therefore, a similar mechanism could be responsible for the chemopreventive effects of PPITC against BOP-induced hamster lung carcinogenesis although this remains to be elucidated in hamsters.

It has been demonstrated that inhibition of the metabolic activation of NNK reduces the formation of $O^6$-methylguanine and consequently decreases the number of neoplasms in the lungs of NNK-treated A/J mice.[2, 3, 5] It has also been shown that the greater inhibitions by arylalkyl isothiocyanates with a longer alkyl chain are quantitatively correlated with decreasing level of $O^6$-methylguanine in the DNA of murine lung tissues.[5] *In vitro* studies have shown that increasing the alkyl chain length enhances the binding affinity of the isothiocyanates to cytochrome P450 isoenzymes, which results in greater inhibition of NNK metabolism.[14] Therefore, it has been suggested that the increased lipophilicity of the longer-chain analogues enhances the binding affinity of the isothiocyanates to cytochrome P450 isoenzymes.[5] However, Hamilton *et al.*[17] found, in an *in vitro* study using hamster liver microsomes, that the superior inhibitory effects of PPITC over PEITC on the NNK-induced mutagenesis were not observed at the 0.1 μmol/plate concentration. Although some species-differences in the inhibition of P450s or the induction of phase II enzymes by these isothiocyanates may exist, PPITC exerted a remarkable chemopreventive action against BOP-induced lung tumorigenesis in hamsters.

In the present study, PPITC did not sufficiently inhibit pancreatic carcinogenesis. The different tissue distributions of PPITC and its different effects on the cytochrome P450 isoenzymes responsible for BOP bioactivation in the hamster pancreas might account, at least in part, for this result. Although the greater chemopreventive potency of isothiocyanates with longer alkyl chains is possibly due to their interaction with the hydrophobic active site of the membrane-bound cytochrome P450 enzymes, other factors such as chemical stability, metabolism, absorption and molecular geometry of these isothiocyanates may also play a role in determining their relative inhibitory potency.[6]

It has been demonstrated that a much longer-chain analogue than PPITC, 6-phenylhexyl isothiocyanate, may be even less toxic than PEITC in rats in a short-term toxicity study (unpublished data). In fact, no toxic changes due to PPITC were found under the present experimental conditions. Therefore, it can be said that PPITC is apparently safe, like PEITC. In conclusion, PPITC could be an efficient chemopreventive agent against lung, but not pancreatic, carcinogenesis in hamsters.

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