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Mechanistic Insights into the Chemopreventive Action of Phenethyl Isothiocyanate against *N*-Nitrosobis(2-Oxopropyl) Amine-Induced Carcinogenesis

In-Seon Lee

Dept. of Food Science and Technology, Keimyung University, Taegu 704-701, Korea

Abstract

The effects of phenethyl isothiocyanate(PEITC) on xenobiotic metabolizing enzymes and cell kinetics in the target organs for *N*-nitrosobis(2-oxopropyl)amine(BOP)-tumorigenicity were investigated in female Syrian golden hamsters in order to gain the mechanistic insights into the chemopreventive action of PEITC against BOP-initiated lung and pancreatic carcinogenesis in hamsters. Hamsters were given BOP subcuteneously(s.c.) and/or PEITC by gavage 2h prior to the BOP treatment. Eight and 24h after the PEITC administration, animals were sacrificed for analyzing P450 isoenzymes, glutathine(GSH), glutathione S-transferase(GST) and cell kinetics. The PEITC pretreatment significantly reduced the hepatic P450 isoenzyme levels such as CYP2B1 and CYP1A1 which were significantly increased by the BOP treatment. However, PEITC did not affect the CYP levels in the pancreas and lung. Interestingly, the PEITC pretreatment rather lowered the hepatic GST and GSH levels, regardless of BOP administration. Proliferating cell nuclear antigen(PCNA)-labeling indices were dose-dependently decreased by PEITC in the pancreas acini and ducts, bronchioles, and renal tubules in which the cell replication was significantly affected by BOP. These results thus suggest that PEITC exerts the chemopreventive effects in hamsters by influencing xenobiotic matabolizing phase I enzymes in the liver and regulating cell kinetics in the target organs.

Key words: phenethyl isothiocyanate, N-nitrosobis(2-oxopropyl)amine

INTRODUCTION

Phenethyl isothiocyanate(PEITC), a natural constituent contained in cruciferous vegetables, has been extensively investgated for its chemopreventive activity against cancer in rats and mice(1). Recently, we have shown that PEITC inhibits lung and panceatic tumorigenesis in hamsters initiated with *N*-nitrosobis(2-oxopropyl) amine(BOP)(2). Principle mechanisms underlying the chemopreventive effects of PEITC are supposedly related to its ability to attenuate DNA alkylation levels induced by chemical carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone(NNK)(1). It has been hypothesized that the decreased levels of DAN alkylation by PEITC are brought about partly by influencing xenobiotic metabolism phase I enzymes, and partly by inducing phase II enzymes(1). In the present study, the influence of PEITC on metabolizing enzymes and cell kinetics was investigated in Syrian golden hamsters given PEITC and/or BOP under the similar condition to our previous long-term bioassays (2,3) in order to elucidate the chemopreventive effects of PEITC during the initiation phase of BOP-induced carcinogenesis in hamsters.

MATERIALS AND METHODS

A total of 60 female Syrian golden hamsters(Japan SLC, Inc., Shizuoka, Japan) 5-weeks old with intial body weights of approximately 80g were used in this experiment. The animals were housed, five per polycarbonate cage, in an air-conditioned room at 23±2°C and 60±5% humidity under a daily cycle of alternating 12h periods of light and darkness. Oriental MF(Oriental Yeast Co., Ltd, Tokyo, Japan) was used as the basal diet. BOP was obtained from Nacalai Tesque(Kyoto, Japan). PEITC(purity>99%) was purchased from Aldrich Chemical Co.(Milwaukee, WI, U.S.A.). Hamsters were given BOP(20mg/kg or 50mg/kg) s.c. and/or PEITC(100μg or 250μg/animal) by gavage 2h prior to the BOP treatment. Eight and 24 hours after the PEITC administration, animals were sacrified. Microsomal proteins were separated by SDS/PAGE on 7.5% polyacryamide gels, transferred to PVDF membranes and proved with goat-rat CYP1A1, 2B1, 2E1, 3A2 and 4A1 sera(Daiichi Pure Chemical Co., Ltd., Tokyo). A rabbit peroxidase

affinity purified antibody to goat IgG(H+L)(BioMarkor ^{TM}bm, Israel) was used as the secondary antibody. GST activity was determined using 1-chloro-2,4-dinitrobenzene as a substrate. GSH levels were measured by the reaction with *o*-phthaladehyde to form a fluorescent product that is activated at 350nm with an emission peak at 420nm. Cell proliferative activity was examined by immunohistochemistry for proliferating cell nuclear antigen(PCNA). The results were statistically evaluated by analysis of variance(ANOVA) and the two-tailed Fisher's exact probability test.

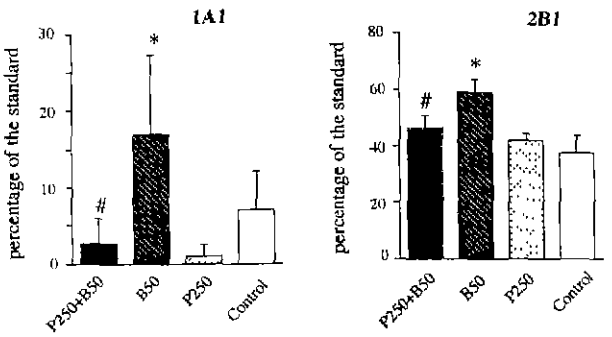

Fig. 1. Hepatic P450 isoenzymes in hamsters treated with PEITC and/or BOP after 8h.
P250, PEITC 250μmol/animal; B50, BOP 50mg/kg
*p<0.05 vs control #P<0.05 vs B50

RESULTS

The PEITC pretreatment significantly reduced the hepatic P450 levels such as CYP2B1 and CYP1A1 which were significantly increased by the BOP treatment although no remarkable induction of CYP 2E1, 3A2 or 4Alwas found in the BOP-treated groups(Fig. 1). PEITC did not affect the P450 isoenzyme levels in the target organs, pancreas and lung. GST activity was significantly decreased($p<0.05$) in each group treated with PEITC and/or BOP as compared to the control value after 8 hours. GSH levels were also significantly decreased($p<0.05$) by the PEITC administration as compared to the BOP alone or non-treated groups after 8 hours although such changes were almost recovered after 24 hours(Fig. 2). Thus, the PEITC pretreatment rather lowered the hepatic GST and GSH levels, regardless of BOP administration. PCNA-labelling indices of the pancreatic acini and ducts, bronchioles, and renal tubules are summarized in Table 1 and Table 2. The percentages of

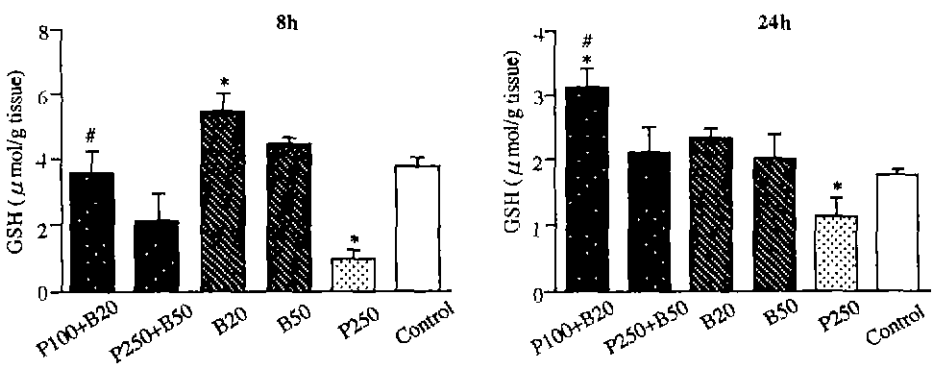

Fig. 2. Hepatic GSH levels in hamsters treated with PEITC and/or BOP after 8h and 24h.
P100, PEITC 100μmol/animal; P250, PEITC 250μmol/animal; B20, BOP 20mg/kg; B50, BOP 50mg/kg
*p<0.05 vs control #P<0.05 vs B20

Table 1. PCNA-labelling indices(Mean±SD) : 8h after PEITC treatment

Organ[1] (cells)	PEITC+ BOP(low)	PEITC+ BOP(high)	BOP (low)	BOP (high)	PEITC	Control
Pancreas(acinar cells)	0.87±0.22$^{\#\#}$	0.67±0.21*	1.68±0.34	1.83±0.42	1.18±0.65	1.21±0.32
Pancreas(duct cells)	1.38±0.94	0.95±0.58**	3.56±2.40	4.23±0.45	1.59±1.73	1.48±0.61
Lung(bronchiolar cells)	0.99±0.65$^{\#}$	0.64±0.65**	3.02±1.19	3.04±0.74	3.03±2.72	2.94±0.73
Liver(bile duct cells)	1.39±1.70	0.57±0.72	1.10±1.43	0.71±0.64	0.72±1.04	0.70±0.89
Kidney(tubule cells)	0.32±0.08$^{\#}$	0.36±0.10**	0.95±0.35	1.34±0.26	0.76±0.51	0.78±0.10

[1]Each group consists of 5 animals
$^{\#}$Significantly different from the BOP(low) group($^{\#}p<0.05$, $^{\#\#}p<0.01$)
Significantly different from the BOP(high) group($p<0.01$, **$p<0.001$)

Table 2. PCNA-labelling indices(Mean±SD) : 24h after PEITC treatment

Organ[1)] (cells)	PEITC+ BOP(low)	PEITC+ BOP(high)	BOP (low)	BOP (high)	PEITC	Control
Pancreas(acinar cells)	0.62±0.35	0.57±0.35**	1.14±0.79	2.18±0.58	0.99±0.54	0.10±0.09
Pancreas(duct cells)	0.59±0.69	1.75±1.43	2.32±2.18	2.23±1.27	1.22±1.11	1.19±0.95
Lung(bronchiolar cells)	0.31±0.28	0.77±1.09*	1.18±0.99	3.70±1.12	2.66±3.58	2.54±1.13
Liver(bile duct cells)	17.11±8.20[#]	9.45±9.82	1.57±1.41	1.75±0.97	6.02±4.47	5.68±3.02
Kidney(tubule cells)	0.09±0.09[#]	0.21±0.19**	0.70±0.47	1.03±0.19	0.40±0.29	0.38±0.21

[1)]Each group consists of 5 animals
[#]Significantly different from the BOP(low) group([#]$p<0.05$, [##]$p<0.01$)
Significantly different from the BOP(high) group($p<0.01$, **$p<0.001$)

PCNA-positive cells were significantly decreased in the BOP/PEITC group as compared to the BOP alone case in a dose-dependent manner.

DISCUSSION

These results thus suggtest that PEITC exerts chemopreventive effects against BOP-induced carcinogenesis in hamsters by influencing xenobiotic matabolizing phase I enzymes in the liver and controlling cell kinetics in the target organs. Regarding phase I enzymes, our data are in good agreement with previous results in rats, mice and hamster treated with NNK (4-6). As suggesed in studies using hamsters(5), a broader spectrum of phase I isoenzymes may be involved in metabolic activation of carcinogenic nitrosamine in hamsters. In the present study, PEITC consistently exerted inhibitory effects on phase I enzymes, regardless of activation by BOP. In contrast to phase I enzymes, the influence of PEITC on GST and GSH was different from the previous experiments using rats in which PEITC induced phase II enzymes such as GST(7,8). Species-specificity may be responsible for this phenomenon. Surprisingly, PEITC effectively inhibited cell proliferative activities increased by BOP treatment even after 8h. It is hypothesized that cell replication in the target organs was enhanced with BOP by stimulating early response genes such as c-myc, c-fos and c-jun (9). Therefore, PEITC may have ability to regulate such genes. Further studies to elucidate these points and the effects of PEITC on DNA methylation in hamsters as an endpoint could warrant our results.

REFERENCES

1. Hecht, S. S. : Chemoprevention by isothiocyanates. *J. Cell. Biochem., Supplement*, **22**, 195(1995)
2. Nishikawa, A., Furukawa, F., Uneyama, C., Ikezaki, S., Tanakamaru, Z., Chung, F.-L., Takahashi, M. and Hayashi, Y. : Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in N-nitrosobis(2-oxopropyl)amine-treated hamsters. *Carcinogenesis*, **17**, 1371(1996)
3. Nishikawa, A., Furukawa, F., Ikezaki, S., Tanakamaru, Z., Chung, F.-L., Takahashi, M. and Hayashi, Y. : Chemopreventive effects of 3-phenylpropyl isothiocyanate on hamster lung tumorigenesis with N-nitrosobis(2-oxopropyl)amine. *Jpn. J. Cancer Res.*, **87**, 122(1996)
4. Guo, Z., Smith, T. J., Eklind, K. I., Chung, F.-L. And Yang, C. S., Structure-activity relationships of arylalkyl isothiocyanate for the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolism and the modulation of xenobiotic-metabolizing enzymes in rats and mice. *Carcinogenesis*, **14**, 1167(1993)
5. Hamilton, S. M., Zhang, Z. and Teel, R. W. : Effects of isothiocyanate alkyl chain-length on hamster liver cytochrome P-450 activity. *Cancer Lett.*, **82**, 217(1994)
6. Yang, C. S., Smith, T. J. and Hong, J. Y. : Cyochrome P-450 enzymes as targets for chemoprevention against chemical carcinogenesis and toxicity : opportunities and limitations. *Cancer Res.*, **54**, 1982(1994)
7. Bogaards, J. J., van Ommen, B., Falke, H. E., Willems, M. I and van Bladeren, P. J. : Glutathione S-transferase subunit induction patterns of Brussels spouts, alkyl isothiocyanate and goitrin in rat liver and small intestinal mucosa : a new approach for the identification of inducing xenobiotics. *Food Chem. Toxicol.*, **28**, 81(1990)
8. Zhang, Y., Talalay, P., Cho, C.-G. and Posner, G. H. : A major inducer of anticarcinogenic protective enzymes from broccoli : isolation and elucidation of structure. *Proc. Natl Acad. Sci.*, **89**, 2399(1992)
9. Shibanuma, M., Kuroki, T. and Nose, K. : Induction of DNA replication and expression of protooncogenes c-myc and c-fos in quiescent Balb/3T3 cells by xanthine/xanthine oxidase. *Oncogenes*, **3**, 17(1988)

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