# ANTIMUTAGENIC EFFECT OF PHENETHYL ISOTHIOCYANATE

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SUMMARY

Using the Ames bacterial mutagenicity test, the comet assay, and an *in vivo* micronucleus test, we investigated the effect of the chemoprotective substance phenethyl isothiocyanate (PEITC) on the mutagenic activity of indirect-acting mutagens and carcinogens aflatoxin B1 (AFB1) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), and direct-acting mutagen and carcinogen N-nitroso-N-methylurea (MNU).

In the Ames test, the antimutagenic activity of PEITC was studied in the concentration range 0.3–300 μg/plate. PEITC at concentrations of 0.3, 3 and 30 μg/plate reduced dose-dependently mutagenicity of AFB1 and IQ in both *S.typhimurium* TA98 and TA100 strains. In the case of the direct mutagen MNU, the antimutagenic effect of PEITC was detected only at concentration of 30 μg/plate in the strain TA100. The PEITC concentration 300 μg/plate was toxic in the Ames test. The 24 h pre-treatment of HepG2 cells with PEITC at concentration 0.15 μg/ml resulted in a significant decrease of DNA breaks induced by MNU at concentrations 0.25 and 0.5 mM. Although a trend towards reduced strand break level were determined also at PEITC concentrations 0.035 and 0.07 μg/ml it did not reach the statistical significance. No effect, however, of PEITC on IQ-induced DNA breaks was observed. Chemopreventive effect of PEITC was revealed also *in vivo*. Pretreatment of mice with PEITC concentrations of 25 and 12.5 mg/kg b.w. administered to mice in three daily doses resulted in reduction of micronucleus formation in mice exposed to all three mutagens under study, with statistically significant effect at concentration of 25 mg/kg.

Results of this study indicate that the strong PEITC antimutagenic properties may have an important role in the prevention of carcinogenesis and other chronic degenerative diseases that share some common pathogenetic mechanisms.

*Key words:* phenethyl isothiocyanate, aflatoxin B1, 2-amino-3-methylimidazo [4,5-]quinoline, N-nitroso-N-methylurea, Ames test, micronucleus test, comet assay

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## INTRODUCTION

The development of tumours is a very complex process in which internal (genetic) factors as well as factors from the external environment participate, the latter being predominant.

The interindividual variability in cancer expression is due to differences in the amount of DNA damage and capacity to repair that damage. Both the amount of DNA damage and the individual repair capacity are influenced by the genetic predisposition (gene polymorphism) and by dietary factors. Dietary factors play also important role in biotransformation and detoxification of genotoxic chemicals.

The tumourigenesis is a multistep process that can be activated by environmental carcinogens, inflamatory agens and tumour promotors. These factors are known to modulate transcription factors, antiapoptotic or proapoptotic proteins, protein kinases, cell cycle proteins, cell adhesion molecules and growth factor signalling pathways. All these molecular targets can be influenced by some plant components that display biochemical and biological activities capable of preventing carcinogensis (1, 2).

One of the rational and effective strategies for chemoprevention is the blockade of DNA damage caused by carcinogenic insult. Complementary strategy is to render organism more resistant to mutagens/carcinogenes and/or to inhibit the progression of the disease (3). The experimental *in vitro* and *in vivo* studies have demonstrated that some dietary components – phytochemicals or compounds that come from edible plants – have inhibitory effects on human cancers suggesting that they may serve as chemopreventive agents (4, 5).

We have also demonstrated the antimutagenic and immunomodulatory effect of substances found in raw vegetables against mutagenicity or immunosuppression caused by $AFB_1$, and pyrolysates of aminoacids (6–10).

Numerous epidemiological studies indicate that Brassica vegetables protect humans against cancer (11). Anti-carcinogenic effect of cruciferous vegetables is attributed to organic isothiocyanates (ITCs) that occur naturally as thioglucoside conjugates (glucosinolates) in a variety of edible cruciferous vegetables. The glucosinolates are hydrolyzed upon chewing or maceration by the enzyme myrosinase to release the isothiocyanate as well as other products. The isothiocyanates are among the most potent anticarcinogenic agents known to reduce occurrence of cancer in different organs including oesophagus, lung and stomach in animal models (reviewed in 12).

One of the most important mechanisms of cancer-chemopreventive activities of isothiocyanates is a modulation of Phase I and Phase II enzymes resulting in reduced activation and/or increased detoxification of carcinogens. Together with modulation of xenobiotic metabolism, modulation of antioxidant defense system may decrease cellular damage by reactive compounds (13–16).

Natural and synthetic isothiocyanates (ITC) have demonstrated cancer-preventive properties in animals treated with chemical carcinogens, including polycyclic aromatic hydrocarbons and nitrosamines (17–19). Phenethyl isothiocyanate (PEITC) reduced the occurrence of experimentally induced tumours in mice, it seems to be very active during tumour initiation (12, 20). Certain ITCs inhibit the proliferation of cultured cancer cell lines by causing cell cycle arrest and/or induction of apoptosis. PEITC inhibits proliferation and induces apoptosis of PC-3 human prostate cancer cells (21), rapidly induces growth inhibition of human promyelocytic leukemia HL60/S cells (22), it is cytotoxic for human breast cancer MCF-7 and mammary epithelial MCF-12A cell lines (23), enhances apoptosis of pulmonary alveolar macrophages and bronchial epithelial cells (24). It inhibits angiogenesis *in vitro* and *ex vivo* even at concentrations achievable by dietary intervention or pharmacologic administration (25). PEITC was proved to be active in the inhibition of the aberrant promotor methylation of GSTP1 gene which is critically inactivated in prostate cancer cells (26). Some molecular targets involved in antiproliferative and proapoptotic activity of PEITC was described by Pullar et al. (27).

Antimutagenic properties of PEITC were detected in several studies. The inhibition of genotoxic effect of dimethylnitroso-amine (DMN) and 2-amino-1methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) by PEITC was detected in the Ames and the micronucleus tests (28), the effect of PEITC on mutagenicity of IQ and other heterocyclic amines in the Ames test (29). Modulation of the clastogenic effect of mitomycine C and cyclophosphamide by PEITC was detected in bone marrow cells of mice (30). The protective effect of isothiocyanates towards N-nitrosamine induced DNA damage was proved in HepG2 cells by the single-cell gel electrophoresis assay (31).

In the present study, we investigated the effect of PEITC on the mutagenicity of three known mutagens/carcinogens: aflatoxin $B_1$ ($AFB_1$), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and N-nitroso-N-methylurea (MNU). Antimutagenic effect of PEITC was detected using the Ames bacterial mutagenicity test, the comet assay on human cell cultures and the micronucleus *in vivo* test.

***Table 1.*** *Effect of PEITC on the mutagenicity of AFB1 in the Ames test (S. typhimurium TA98, TA100)*

| AFB1+PEITC dose (mg/plate) | *S. typhimurium* TA98+S9 | | | *S. typhimurium* TA100+S9 | | |
|---|---|---|---|---|---|---|
| | No. of revertants | ±SD | % of inhibition | No. of revertants | ±SD | % of inhibition |
| 10+0 | 908 | 109 | | 1,377 | 63 | |
| 10+0.3 | 941 | 138 | +4 | 1,353 | 57 | -2 |
| 10+3 | 624* | 50 | -31 | 965** | 64 | -30 |
| 10+30 | 52** | 34 | -94 | 161** | 82 | -88 |
| 10+300 | 0 | | - | 0 | | - |
| 1+0 | 440 | 106 | | 852 | 80 | |
| 1+0.3 | 309* | 52 | -30 | 536** | 93 | -37 |
| 1+3 | 154** | 48 | -65 | 372** | 34 | -56 |
| 1+30 | 39** | 23 | -91 | 94** | 29 | -89 |
| 1+300 | 0 | | - | 0 | | - |
| 0.1+0 | 120 | 12 | | 391 | 32 | |
| 0.1+0.3 | 102 | 15 | -15 | 259** | 57 | -34 |
| 0.1+3 | 76** | 17 | -37 | 199** | 25 | -49 |
| 0.1+30 | 17** | 8 | -86 | 69** | 21 | -82 |
| 0.1+300 | 0 | | - | 0 | | - |
| Control (DMSO) | 23 | 3 | | 110 | 14 | |
| 0+0.3 | 26 | 5 | | 102 | 8 | |
| 0+3 | 30 | 3 | | 103 | 12 | |
| 0+30 | 21 | 5 | | 79 | 19 | |
| 0+300 | 0 | | | 0 | | |

*Statistically significant difference between the sample with PEITC and mutagen and the sample with mutagen alone: $p<0.05$
**Statistically significant difference between the sample with PEITC and mutagen and the sample with mutagen alone: $p<0.01$
SD: standard deviation

***Table 2.*** *Effect of PEITC on the mutagenicity of IQ in the Ames test (S. typhimurium TA98, TA100)*

| IQ+PEITC dose (mg/plate) | *S. typhimurium* TA98+S9 | | | *S. typhimurium* TA100+S9 | | | |
|---|---|---|---|---|---|---|---|
| | No. of revertants | ±SD | % of inhibition | dose (g/plate) | No. of revertants | ±SD | % of inhibition |
| 0.1+0 | 1,080 | 44 | | 10+0 | 1,710 | 216 | |
| 0.1+0.3 | 764** | 85 | -29 | 10+0.3 | 1,517 | 128 | -11 |
| 0.1+3 | 238** | 38 | -78 | 10+3 | 706** | 148 | -59 |
| 0.1+30 | 13** | 4 | -99 | 10+30 | 98** | 35 | -94 |
| 0.1+300 | 0 | | - | 10+300 | 0 | | - |
| 0.01+0 | 310 | 35 | | 1+0 | 648 | 103 | |
| 0.01+0.3 | 220** | 51 | -29 | 1+0.3 | 432** | 27 | -33 |
| 0.01+3 | 75** | 10 | -76 | 1+3 | 225** | 77 | -65 |
| 0.01+30 | 23** | 19 | -93 | 1+30 | 87** | 27 | -87 |
| 0.01+300 | 0 | | - | 1+300 | 0 | | - |
| 0.001+0 | 97 | 9 | | 0.1+0 | 206 | 24 | |
| 0.001+0.3 | 58** | 9 | -40 | 0.1+0.3 | 154** | 7 | -25 |
| 0.001+3 | 32** | 5 | -67 | 0.1+3 | 110** | 25 | -47 |
| 0.001+30 | 25** | 12 | -74 | 0.1+30 | 82** | 15 | -60 |
| 0.001+300 | 0 | | - | 0.1+300 | 0 | | - |
| Control (DMSO) | 25 | 5 | | Control (DMSO) | 93 | 19 | |
| 0+0.3 | 28 | 4 | | 0+0.3 | 91 | 9 | |
| 0+3 | 29 | 4 | | 0+3 | 85 | 8 | |
| 0+30 | 20 | 7 | | 0+30 | 83 | 3 | |
| 0+300 | 0 | | | 0+300 | 0 | | |

**Statistically significant difference between the sample with PEITC and mutagen and the sample with mutagen alone: p<0.01
SD: standard deviation

## MATERIAL AND METHODS

### The Ames Test

For the evaluation of antimutagenic effect of PEITC *in vitro*, the Ames test with *Salmonella typhimurium* TA98 and TA100 (32, 33, 34) was used. Mutagenic substances were used at the following concentrations: $AFB_1$ at concentrations of 10 μg, 1 μg and 0.1 μg per plate in both strains, TA98 and TA100, IQ at concentrations of 0.1 μg, 0.01 μg and 0.001 μg per plate in the strain TA98, at concentrations of 10 μg, 1 μg and 0.1 μg in the strain TA100, MNU at concentrations of 1000 μg, 100 μg and 10 μg only in the strain TA100 as these concentrations had no effect in the strain TA98. Each concentration of each mutagen was combined with four different concentrations of PEITC (300 μg, 30 μg, 3 μg, and 0.3 μg per plate). All chemicals were diluted in DMSO. The S9 fraction of liver homogenate from male Wistar albino rats induced by a mixture of polychlorinated biphenyls Delor 103 was used for metabolic activation of indirect mutagens (33). Each combination of mutagen and antimutagen was tested in two separate experiments with three plates in each experiment.

The percentage of inhibition of the mutagenicity was calculated by the formula:

$$\frac{\text{No. of revertants of mutagen} - \text{No. of revertants of a mixture of mutagen and PEITC}}{\text{No. of revertants of mutagen}} \times 100$$

Statistical evaluation was carried out using Student's t-test.

### The Micronucleus Test

*In vivo* bone marrow micronucleus test was carried out on male Balb C mice, each weighting 20–24 g (BIOTEST, Konárovice). The animals were housed under standard conditions and were fed with a commercial granulated mixture for laboratory rodents. Animals were divided into group of 6 mice each.

PEITC was applied *per os* at the doses of 25 mg/kg and 12.5 mg/kg of the murine body weight for three consecutive days by gavage. The mutagens were administered at one dose on the third day, 1–1.5 h after the application of PEITC ($AFB_1$ at the dose of 1 mg/kg b.w., IQ at the dose of 20 mg/kg b.w., MNU at the dose of 50 mg/kg b.w.). All substances were dissolved in 7%

DMSO and applied in volumes of 0.1 ml/10g b.w. The control group obtained equal amounts of the solvent (7% DMSO).

The mouse bone marrow micronucleus test was carried out according to Schmid (35). A total of 1,000 polychromatophilic erythrocytes were scored per animal. Each experiment was performed twice. The statistical evaluation was carried out using the Student's t-test.

## The Comet Assay

The amount of single strand DNA breaks (SSB) induced in HepG2 cells pretreated with PEITC and treated subsequently with MNU or IQ was measured by the comet assay. The alkaline version of the comet assay according to Singh et al. (36) modified by Collins et al. (37) was used. Briefly, cells embedded in agarose on a microscope slide were lysed in high-salt buffer containing non-ionic detergent and left to unwind the DNA in alkaline electrophoresis solution. During the following electrophoresis the DNA migrates to the anode. After the staining of cells with fluorescent dye (ethidium bromide, Sigma) cells in fluorescent microscope resembles comets. The amount of DNA in the tail of comets reflects the amount of the DNA damage (SSB – single strand breaks). The per cent of DNA in the tail was converted to the number of SSB/109 daltons using the calibration of the method by X- ray irradiation (38). 50 cells were scored per experimental point in each experiment. The statistical significance of the difference between the DNA damage in cells treated with carcinogen only and the cells pretreated also by PEITC was tested using the Mann-Withney test.

## RESULTS

## The Ames Test

In the Ames test PEITC strongly reduced the mutagenicity of all concentrations of two indirect mutagens $AFB_1$ and IQ, and the mutagenicity of direct mutagen MNU at the dose of 30 μg/plate. The highest dose of 300 μg PEITC/plate was fully toxic in combination with all mutagens and also in the sample with PEITC used alone. Other concentrations of PEITC (30, 3 and 0.3 μg/plate) did not reveal any mutagenic activity when used alone. The concentration of 30 μg PEITC/plate seemed to be sligtly toxic because the number of revertants fell down below the controls.

The mutagenicity of $AFB_1$ (concentrations of 10, 1 a 0.1 μg/plate in both strains of *Salmonella typhimurium* TA98 and TA100) was significantly reduced by the dose of 30 μg PEITC/plate by 94, 91 and 86% in the strain TA98, and by 88, 89 and 82% in the strain TA100 (Table 1). The concentration of 3 μg/plate significantly decreased the effect of three concentrations of $AFB_1$ by 31, 65 a 37% in the strain TA98 and by 30, 56 and 49% in the strain TA100. The lowest PEITC concentration (0.3 μg/plate) had not influence on the highest concentration of $AFB_1$ – 10 μg/plate in both strains, but mutagenicity of two lower concentrations of mutagen was reduced by this dose within the range from 15 to 37%. 30 μg of PEITC in combination with 0.1 μg of $AFB_1$ reduced numbers of revertants to the levels below control numbers of revertants in both strains thus revealed possibly slight toxicity of this concentration for cells.

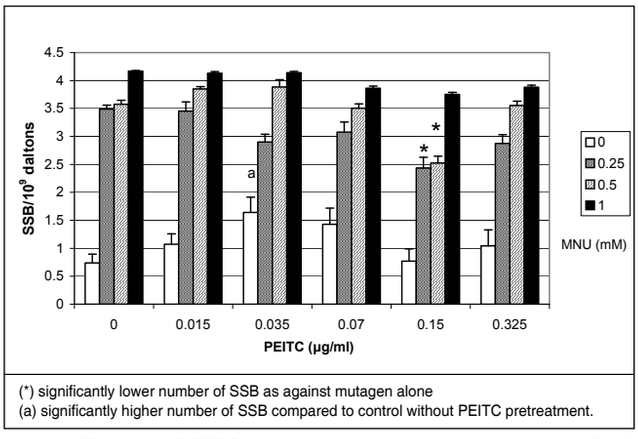
All three concentrations of PEITC in combination with mutagen IQ revealed statistically significant dose dependent antimutagenic activity in both strains TA98 and TA100, except combinations of 10 μg IQ/plate and 0.3 μg PEITC/plate in the strain TA100 (Table 2). The dose of 30 μg PEITC/plate decreased the mutagenicity of different concentrations of IQ (0.1, 0.01 a 0.001 μg/plate) by 99, 93 and 74% in the strain TA98, the mutagenicity of 10, 1 a 0.1 μg of IQ/plate by 94, 87 and 60% in the strain TA100. The reduction of mutagenity came near to control levels of revertants and in the case of 0.1 μg of IQ in the strain TA98 even below this levels. The dose of 3 μg/plate reduced the effect of the same concentrations of IQ by 78, 76 a 67% in the strain TA98 and by 59, 65 a 47% in TA100. The lowest concentration of 0.3 μg/plate decreased the mutagenicity by 29, 29 and 40% in the strain TA98 and by 11, 33 and 25% in TA100.

30 μg/plate of PEITC strongly inhibited the mutagenicity of the direct mutagen MNU, used at the concentrations of 1000, 100 a 10 μg/plate without metabolic activation, by 80, 90 a 78% in the strain TA100 (Table 3). But lower doses of PEITC were not effective. Only in a combination with two lower mutagen concentrations these doses inhibited slightly and nonsignificantly the mutagenicity of MNU. The lowest concentration of PEITC (0.3 μg/plate) was not antimutagenic in combinations with 1,000 a 100 μg/plate of MNU at all.

***Table 3.*** *Effect of PEITC on the mutagenicity of MNU in the Ames test (S. typhimurium TA100)*

| MNU+PEITC dose (mg/plate) | S. typhimurium TA100 | | |
|---|---|---|---|
| | No. of revertants | ±SD | % of inhibition |
| 1,000+0 | 1,324 | 270 | |
| 1,000+0.3 | 1,326 | 249 | 0 |
| 1,000+3 | 1,312 | 199 | -1 |
| 1,000+30 | 267** | 168 | -80 |
| 1,000+300 | 0 | | - |
| 100+0 | 1,594 | 157 | |
| 100+0.3 | 1,587 | 259 | 0 |
| 100+3 | 1,441 | 186 | -10 |
| 100+30 | 159** | 145 | -90 |
| 100+300 | 0 | | - |
| 10+0 | 300 | 43 | |
| 10+0.3 | 279 | 53 | -7 |
| 10+3 | 237 | 52 | -21 |
| 10+30 | 65** | 12 | -78 |
| 10+300 | 0 | | - |
| Control (DMSO) | 83 | 11 | |
| 0+0.3 | 86 | 5 | |
| 0+3 | 94 | 3 | |
| 0+30 | 60 | 16 | |
| 0+300 | 0 | | |

** Statistically significant difference between the sample with PEITC and mutagen and the sample with mutagen alone: $p<0.01$
SD: standard deviation




(*) significantly lower number of SSB as against mutagen alone
(a) significantly higher number of SSB compared to control without PEITC pretreatment.

***Fig. 1.*** *Effect of PEITC on the induction of single strand DNA breaks in HepG2 cells pretreated with PEITC for 24 h and then treated with MNU for 1 h.*

## The Micronucleus Test

In the micronucleus test all three mutagens revealed significant mutagenic activities. The numbers of micronuclei in the animals influenced by PEITC alone did not differ from those of the control group. On oral application of three doses of 25 mg/kg b.w. of PEITC before one dose of $AFB_1$ (1 mg/kg b.w.), the number of micronuclei in polychromatophilic erythrocytes was lower in a statistically significant degree in comparison with the laboratory mice treated with $AFB_1$ alone. The dose of 12.5 mg/kg b.w. decreased the number of micronuclei induced by $AFB_1$ only insignificantly. A dose dependent antimutagenic effect was detected on the application of the IQ mutagen. Both concentrations of PEITC (three doses of 25 or 12.5 mg/kg b.w.) in combination with one dose of IQ (20 mg/kg b.w.) significantly reduced its mutagenic effect. Similarly, the treatment of mice with the combination of the same doses of PEITC and one dose of MNU (50 mg/kg b.w.) led to a reduction of the number of micronuclei induced by MNU with significant reduction at the dose 25 mg/kg b.w. The results are presented in Table 4.

## The Comet Assay

HepG2 cells were treated with diferent concentrations of PEITC for 24 h and then immediately for 1 hour with MNU or for 2 hours with IQ. As it is evident from the Fig.1, PEITC caused a dose dependent decrease of single strand DNA breaks (SSB) in cells treated with 0.25 or 0.5 mM MNU. The decrese was clearly visible begining with the concentration 0.035 µg/ml of PEITC, being statistically significant at 0.15 µg/ml. The higher concentration of PEITC did not show the inhibiting effect on the SSB induction, because those concentrations of PEITC caused significant induction of SSB itselves. A slight increase of SSBs in cultures treated with PEITC only was observed also at lower concentrations, being singnificant at 0.035 µg/ml. In similar experiments we have not observed any effect of PEITC on the induction of SSB by IQ (data not shown).

## DISCUSSION

In our work we revealed the strong dose-dependent inhibition effect of PEITC on the mutagenicity of two indirect mutagens $AFB_1$ and IQ in the Ames test. On the contrary to authors who

***Table 4.*** *Impact of PEITC pretreatment on the clastogenic effect of AFB1, IQ and MNU in vivo (mice pretreated with PEITC at doses 25 mg/kg and 12.5 mg/kg b.w. for three consecutive days before administration of mutagens)*

| Substances studied | No. of micronuclei ± SD | K |
|---|---|---|
| Control – 7% DMSO | 0.5±0.6 | |
| PEITC – 3x12.5 mg/kg | 0.4±0.5 | 0.8 |
| PEITC – 3x25 mg/kg | 0.4±0.5 | 0.8 |
| Control – 7% DMSO | 0.8±1.0 | |
| AFB1 – 1 mg/kg | $5.0^{aa}$±1.4 | 6.3 |
| PEITC + AFB1 – 3x12.5 mg/kg + 1 mg/kg | $3.7^{aa}$±1.2 | 4.6 |
| PEITC + AFB1 – 3x25 mg/kg + 1 mg/kg | 1.7**±0.5 | 2.1 |
| Control – 7% DMSO | 0.7±0.8 | |
| IQ – 20 mg/kg | $4.3^{aa}$±0.8 | 6.1 |
| PEITC + IQ – 3x12.5 mg/kg + 20 mg/kg | $2.7^{**aa}$±0.8 | 3.9 |
| PEITC + IQ – 3x25 mg/kg + 20 mg/kg | $1.8^{**a}$±0.8 | 2.6 |
| Control – 7% DMSO | 0.5±0.5 | |
| MNU 50 mg/kg | $14.7^{aa}$±4.5 | 29.4 |
| PEITC + MNU – 3x12.5 mg/kg + 50 mg/kg | $12.7^{aa}$±0.5 | 25.4 |
| PEITC + MNU –3x25 mg/kg + 50 mg/kg | $8.3^{*aa}$±1.2 | 16.6 |

** significantly lower number of micronuclei as against mutagen alone, p<0.01
* significantly lower number of micronuclei as against mutagen alone, p<0.05
aa significantly lower number of micronuclei as against control, p<0.01
a significantly lower number of micronuclei as against control, p<0.05
SD – standard deviation
K – ratio of induced to spontaneous micronucleus levels

describe strong genotoxic effect of PEITC *in vitro* and slight genotoxic effect *in vivo* (39) we did not proved any mutagenic activity of this compound in the Ames and the micronucleus tests, but concentration of 300 μg/plate was toxic in the Ames test. The antimutagenic effect against the direct mutagen MNU was detected only at concentration of 30 μg/plate in the Ames test. In some experiments the concentration of 30 μg/plate of PEITC revealed antiproliferative or slight bacteriotoxic effects, because the number of revertants was reduced below the control levels. Also *in vivo* PEITC reduced a number of micronuclei in mice exposed to all three mutagens. Similar antimutagenic activity of PEITC on mutagenicity of heterocyclic amines in the Ames test was achieved by Hamilton and Teel (29). Knasmüller *et al.* (28) measured the mutagenic activity of dimethylnitrosamine and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine after metabolic activation with S9 homogenate from organs of PEITC treated mice and also proved a reduction of mutagenicity.

Antimutagenic and anticarcinogenic effects of PEITC are caused by the influence on a metabolic activation of mutagen by the inhibition of the level and/or activity of CYP enzymes and also by the effect on detoxification enzymes (15, 29, 40). PEITC has also the antiproliferative effect and induces apoptosis (24, 41, 42). Described bacteriotoxic effect of ITCs (24, 43) suggests that the stimulation of apoptosis by chemopreventive agents may often reflect occurrence of toxic effects at high doses.

In our experiments strong bacteriotoxic effect was proved at a concentration of 300 μg PEITC/plate in the Ames test. Antiproliferative or slightly cytotoxic effect of a concentration of 30 μg/plate of PEITC was probably the main reason for the antimutagenicity against the direct mutagen MNU. The strong antimutagenic effect against the indirect mutagens $AFB_1$ and IQ is possibly result of influences on metabolic activation and detoxification of mutagens combined with antiproliferative effects at higher concentrations of PEITC, at lower concentrations of PEITC the effect on mutagen metabolism is predominant.

In contrast to findings of Arranz *et al*. (31), who described the PEITC-caused reduction of SSB induced by N-nitrosamine in HepG2 cells, we have not seen any PEITC- caused reduction of SSB induced by IQ. Our results are in accordance with findings of García *et al*. (44), who found a weak protective effect of PEITC towards N-nitrosodibutylamine or N-nitrosopiperidine oxidative DNA damage in the single cell gel electrophoresis (SCGE)/HepG2 assay. On the other hand, rather surprising was our finding that PEITC is able to reduce the induction of DNA damage by directly acting alkylating agent MNU. This, to our knowledge, has not been described, and it seems to support the view, that PEITC is able to induce the phase II detoxification enzymes (45, 46).

PEITC and other isothiocyanates appear to have many favourable properties including antimutagenic effect and that make them attractive for other study and development as chemopreventive agents for human cancer and some other human pathologies that share some common pathogenetic mechanisms.

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