SHORT COMMUNICATION

Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate

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Epidemiological studies have linked consumption of broccoli to a reduced risk of colon cancer in individuals with the glutathione S-transferase M1 (GSTM1) null genotype. GSTs are involved in excretion and elimination of isothiocyanates (ITCs), which are major constituents of broccoli and other cruciferous vegetables and have cancer chemopreventive potential, so it is speculated that ITCs may play a role in protection against human colon cancer. However, there is a lack of data from animal studies to support this. We carried out a bioassay to examine whether sulforaphane (SFN) and phenethyl isothiocyanate (PEITC), major ITCs in broccoli and watercress, respectively, and their corresponding N-acetyl cysteine (NAC) conjugates, show any chemopreventive activity towards azoxymethane (AOM)-induced colonic aberrant crypt foci (ACF) in F344 rats. Groups of six male F344 rats were treated with AOM subcutaneously (15 mg/kg body wt) once weekly for 2 weeks. SFN and PEITC and their NAC conjugates were administered by gavage either three times weekly for 8 weeks (5 and 20 µmol, respectively) after AOM dosing (post-initiation stage) or once daily for 3 days (20 and 50 µmol, respectively) before AOM treatment (initiation stage). The bioassay was terminated on week 10 after the second AOM dosing and ACF were quantified. SFN, SFN-NAC, PEITC and PEITC-NAC all significantly reduced the formation of total ACF from 153 to 100–116 (P < 0.01) and multicrypt foci from 52 to 27–38 (more than four crypts/focus; P < 0.05) during the post-initiation treatment. However, only SFN and PEITC were effective during the initiation phase, reducing the total ACF from 153 to 109–115 (P < 0.01) and multicrypt foci from 52 to 35 (more than four crypts/focus; P < 0.05). The NAC conjugates were inactive as anti-initiators against AOM-induced ACF. These findings provide important laboratory evidence for a potential role of SFN and PEITC in the protection against colon cancer.

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; BITC, benzyl isothiocyanate; CYP, cytochrome P450; GST, glutathione S-transferase; ITCs, isothiocyanates; NAC, N-acetylcysteine; PEITC, phenethyl isothiocyanate; SFN, sulforaphane.
Table I. Effects of SFN and PEITC on the formation of aberrant crypt foci induced by AOM

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose of ITC compounds (µmol)</th>
<th>Average body weight at termination</th>
<th>Number of aberrant crypt foci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;Four crypts</td>
</tr>
<tr>
<td>1. AOM</td>
<td>–</td>
<td>310</td>
<td>52</td>
</tr>
<tr>
<td>2. AOM→SFN</td>
<td>5</td>
<td>301</td>
<td>30 (42)bc</td>
</tr>
<tr>
<td>3. AOM→SFN–NAC</td>
<td>20</td>
<td>297</td>
<td>31 (40)c</td>
</tr>
<tr>
<td>4. AOM→PEITC</td>
<td>5</td>
<td>306</td>
<td>27 (48)ec</td>
</tr>
<tr>
<td>5. AOM→PEITC–NAC</td>
<td>20</td>
<td>313</td>
<td>38 (27)f</td>
</tr>
<tr>
<td>6. SFN→AOM</td>
<td>20</td>
<td>310</td>
<td>35 (33)df</td>
</tr>
<tr>
<td>7. SFN–NAC→AOM</td>
<td>50</td>
<td>304</td>
<td>44 (15)</td>
</tr>
<tr>
<td>8. PEITC→AOM</td>
<td>20</td>
<td>307</td>
<td>35 (33)df</td>
</tr>
<tr>
<td>9. PEITC–NAC→AOM</td>
<td>50</td>
<td>303</td>
<td>74 (42)df</td>
</tr>
<tr>
<td>10. SFN</td>
<td>5</td>
<td>309</td>
<td>0</td>
</tr>
<tr>
<td>11. SFN–NAC</td>
<td>20</td>
<td>312</td>
<td>0</td>
</tr>
<tr>
<td>12. PEITC</td>
<td>5</td>
<td>333</td>
<td>0</td>
</tr>
<tr>
<td>13. PEITC–NAC</td>
<td>20</td>
<td>301</td>
<td>0</td>
</tr>
<tr>
<td>14. Control</td>
<td>–</td>
<td>320</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: All ITC compounds were administered during the post-initiation phase in groups 2–5 and during the initiation phase in groups 6–9.

*Significantly different from group 1 ($P < 0.01$).
**Significantly different from group 1 ($P < 0.0001$).
†Significantly different from group 1 ($P < 0.001$).
‡Significantly different from group 1 ($P < 0.05$).

Fig. 1. Structures of SFN and PEITC and their NAC conjugates.

For the induction of colon tumor (16), SFN and PEITC in corn oil and NAC conjugates in saline (10% dimethylsulfoxide) were given by gavage. The high cost of SFN prohibited administration of the compound in the diet. For the post-initiation treatment, groups 2–5 were given 5 µmol SFN or PEITC or 20 mmol of the NAC conjugates, three doses each week for 8 weeks, beginning two days after the last dose of AOM. The higher doses of the NAC conjugates were given because of their gradual release of parent compounds and lower toxicity. For the assay of anti-initiation activity, groups 6–9 were treated with three doses of ITC compounds (20 µmol ITC or 50 µmol ITC–NAC) once daily; the last dose was given 2 h before AOM dosing. This dosing regimen was repeated during the second week of AOM dosing. This pretreatment protocol was adapted based on earlier studies on mammary tumor and lung tumor models (17,18). Groups 10–13 were treated with ITCs (5 µmol/dose) or the conjugates (20 µmol/dose) only, three times weekly for 8 weeks. The bioassay was terminated at week 10 after the second AOM treatment. All animals were killed by CO₂ asphyxiation. The colons were excised, fixed in buffered formalin and processed for microscopic examination; ACF were recorded using a standard procedure described previously (19). ACF were distinguished from the surrounding normal crypts by their increased size, significantly increased distance from lamina to basal surface of cells and the easily discernible pericryptal zone. For statistical analysis, means were compared among the groups using one-way analysis of variance (ANOVA) followed by Fisher’s protected t-test.

No significant differences in body weights were seen in any of the treated groups compared with the control group, indicating that doses of ITCs and the conjugates used did not cause overt toxicity (Figure 2). This bioassay showed that post-treatment with SFN and PEITC by oral administration at 5 µmol three times weekly for 8 weeks and their NAC conjugates at 20 µmol by the same regimen inhibited formation of ACF. These treatments reduced the total number of ACF from 153 to 100–116 ($P < 0.01$) and reduced the number of multicytotic foci (more than four crypts/focus) from 52 to 27–37 ($P < 0.05$) as shown in Table I (groups 1–5). Because the ITC conjugates are presumably less toxic than the parent ITCs, the doses of the conjugates were four times that of SFN and PEITC (5,20). However, we did not find significant differences in the inhibition of ACF between the ITCs and their NAC conjugates, suggesting that the conjugates themselves are not as active. Similar dose–effect relationships were observed previously between parent ITCs and their conjugates towards the inhibition of lung tumorigenesis (13). These results, together with those from our earlier studies (21,22), suggest that ITC conjugates render their inhibitory activity in part by deconjugation to the parent ITCs. Although the mechanism of inhibition of ACF at the post-initiation phase is not currently clear, studies have shown that PEITC and related ITCs induce p53-dependent or c-Jun kinase-mediated apoptosis in cultured tumor cells (23–25). More recently it has been reported that SFN induces cell cycle arrest in HT29 human colon cancer cells (26). The growth arrest, followed by cell death via
apoptosis, appeared to be associated with expression of cyclins A and B1. In addition, NAC produced by deconjugation is a known antioxidant and has been recently shown to inhibit mouse fibroblast cell proliferation by blocking cell in G1 phase (27). All these activities could have contributed to the inhibition of ACF formation during post-initiation.

As shown previously by us and others, pretreatment of animals with PEITC and other related ITCs blocked chemical-induced tumorigenicity by inhibiting cytochrome P450 (CYP) enzymes responsible for the activation of carcinogens, and consequently reducing DNA damage (28–30). Using the pretreatment regimen, we investigated the effects of SFN and PEITC on AOM-induced colonic ACF formation in Fischer rats. Pretreatment with SFN or PEITC significantly decreased the total number of ACF from 153 to 109 \((P < 0.001)\) or 115 \((P < 0.01)\), respectively, and multicrypt foci (more than four crypts) from 52 to 35 \((P < 0.05)\) for both compounds (Table I). We have previously shown that PEITC and PEITC–NAC inhibit \(N,N\)-dimethylnitrosamine (NDMA) demethylase (CYP2E1) in rat liver microsomes (21). Like NDMA, AOM is metabolically activated by CYP2E1 to methyazoxymethanol, which can yield a DNA methylating species (31). Thus, inhibition of CYP2E1 by these agents in the rat liver may constitute an important mechanism of inhibition, because CYP enzyme activity in colonic tissue is low compared with that in liver (32). SFN–NAC also reduced total ACF to 120 \((P < 0.01)\); however, it had no significant effect on multicrypt foci (those with more than four crypts/focus; 15% inhibition). PEITC–NAC, on the other hand, appeared to enhance ACF formation [total number of ACF was 198 \((P < 0.001)\); number of multicrypt foci was 74 \((P < 0.05)\)] (Table I). The adverse effect caused by PEITC–NAC is somewhat unexpected in view of the inhibition by its parent ITC. At present, it is not clear why there is such a sharp contrast in ACF formation between PEITC–NAC and the other ITC compounds studied here. Previously, it has been reported that PEITC given in the diet at 0.1% for 32 weeks appeared to promote bladder cancer development in rats pretreated with carcinogens (33). This dose is considerably higher than the doses used in the present study. Phenylhexyl ITC, a synthetic homolog of PEITC, is a potent inhibitor of lung tumorigenesis in rats and mice, whereas it enhanced colon and esophagus tumorigenesis in rats, possibly due to its tissue cytotoxicity at the dose level studied (34–37). These results illustrate the importance of choosing the appropriate dose range for specific tissues in chemoprevention studies.

Evidence from epidemiological studies suggests an association between consumption of cruciferous vegetables and a reduced risk of colon cancer (38). Although the beneficial effects may result from the combined effects of a number of compounds in these vegetables, the exact role of each compound that contributes to the protective effects needs to be identified more clearly. Ample data from laboratory animal studies have shown that ITCs, major constituents of cruciferous vegetables, are promising chemopreventive agents against cancers at various sites, including lung, esophagus, liver, mammary, pancreas and bladder (3,28,39). These laboratory results support a potential role of dietary ITCs in reducing the risk of certain human cancers. Considering the natural abundance of SFN and PEITC in broccoli and watercress [it is estimated that an individual’s intake of SFN or PEITC after ingesting 100 g (dry weight) of broccoli or watercress can range from 50 to 200 µmol (40–43)], and their potential as chemopreventive agents, it is surprising that little is known about the effects of these agents on colon tumorigenesis. A lower homolog of PEITC, benzyl ITC (BITC) has been shown to reduce colon tumor incidence in AOM treated rats during the initiation phase, but not during the post-initiation phase (44). Another short-term study, however, has reported that both PEITC and BITC given in the diet at similar doses throughout the bioassay did not affect ACF formation (12). By contrast, the present study has demonstrated that both PEITC and SFN inhibit colonic ACF, independent of whether they are administered before or after exposure to carcinogen. These data, thus, support a potential protective role of SFN and PEITC in colon cancer and are consistent with the epidemiological observation that consumption of certain cruciferous vegetables reduces the risk of colon cancer in individuals with GSTM1 null genotype. These findings warrant further investigations of the mechanisms of action and preclinical efficacy of these agents.

Acknowledgements

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References


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