Anticarcinogenic Activities of Organic Isothiocyanates: Chemistry and Mechanisms

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Abstract

Organic isothiocyanates block the production of tumors induced in rodents by diverse carcinogens (poly cyclic aromatic hydrocarbons, azo dyes, ethionine, N-2-fluorenylacetamide, and nitrosamines). Protection is afforded by α-naphthyl-, β-naphthyl-, phenyl-, benzyl-, phenethyl-, and other arylalkyl isothiocyanates against tumor development in liver, lung, mammary gland, stomach, and esophagus. Many isothiocyanates and their glucosinolate precursors (β-thioglucoside, N-hydroxysulfate) occur naturally and are found in a variety of plants consumed by humans, e.g., cruciferous vegetables. Nevertheless, the possible contributions of isothiocyanates and glucosinolates to the well recognized protective effects against cancer of high consumption of vegetables are unclear. The anticarcinogenic effects of isothiocyanates appear to be mediated by tandem and cooperating mechanisms: (a) suppression of carcinogen activation by cytochromes P-450, probably by a combination of down-regulation of enzyme levels and direct inhibition of their catalytic activities, which thereby lower the levels of ultimate carcinogens formed; and (b) induction of Phase 2 enzymes such as glutathione transferases and NAD(P)H:quinone reductase, which detoxify any residual electrophilic metabolites generated by Phase 1 enzymes and thereby destroy their ability to damage DNA. Since isothiocyanates block carcinogenesis by dual mechanisms and are already present in substantial quantities in human diets, these agents are ideal candidates for the development of effective chemoprotection of humans against cancer.

Introduction

Organic isothiocyanates (R—N=C=S), also known as mustard oils, are widely distributed in plants, many of which are consumed by humans. They are responsible for the pungent and acrid flavor and odor of condiments such as mustard and horseradish and the familiar biting taste that develops when some cruciferous vegetables are eaten. The designation “mustard oil” originates from the flavor of mustard seeds which is largely due to the presence of other potentially confounding substances. The hydrolysis of glucosinolates by myrosinase. The quantities and nature of these products are variable and appear to be controlled by the chemical nature of the glucosinolate, the pH, and the origin and multiplicity of the myrosinases. A full discussion of the formation of these products is provided by Fenwick et al. (2).

Our interest in isothiocyanates stemmed from the observations that several members of this family of substances could block the toxic and neoplastic effects of a wide variety of chemical carcinogens (6). Furthermore, many isothiocyanates are monofunctional inducers of Phase 2 enzymes (7, 8), a property that is associated with protection against chemical carcinogenesis (9). Recently, a very potent isothiocyanate inducer (sulforaphane) has been isolated from broccoli (10).

These observations point to the potential importance of isothiocyanates as chemoprotectors against cancer in humans.

This review focuses on the metabolism of isothiocyanates and on the mechanisms of their anticarcinogenic effects. We discuss only experiments in which pure isothiocyanates have been used and omit corroborating information obtained with plants and their extracts, because of the presence of other potentially confounding substances.

Chemical Properties and Metabolism of Isothiocyanates

Chemical Reactivity and Spectroscopic Properties. The highly electrophilic central carbon atom of the —N=C=S group reacts rapidly, and under mild conditions with oxygen-, sulfur-, or nitrogen-centered nucleophiles to give rise to carbamates, thiocarbamates, or thiourea derivatives, respectively.

These reaction products have been useful for the spectroscopic identification and characterization of isolated isothiocyanates. The —NCS group of isothiocyanates absorbs UV light with low intensity near 240 nm (aP about 1000 M−1 cm−1). Reactions of isothiocyanates with monothiols at pH 7–9 yield thiocarbamates (carbamate thioesters) that show markedly enhanced UV absorption intensity (aP 10,000 M−1 cm−1) and a characteristic maximum at 270 nm with a broad shoulder near 250 nm (11–13). Zhang et al. (13) have reported recently that isothiocyanates react with vicinal dithiols, such as 2,3-dimercaptopropanol or ethane-1,2-dithiol, to give rise initially to the expected monothiocarbamates. However, upon further incubation these primary products undergo attack by the free thiol group on the electrophilic carbon, resulting in a cyclization reaction with release of the free amine (13, 14). Thus the reaction of any R-NCS with ethane-1,2-dithiol, 2,3-dimercaptopropanol, and 1,2-benzenedithiol gives rise to ethylene trithiocarbonate (1,3-dithiolane-2-thione, λmax 316 nm; aP 16,500 M−1 cm−1), 4-hydroxymethyl-1,3-dithiolane-2-thione (λmax 316 nm; aP 16,400 M−1 cm−1), and 1,3-benzodithiole-2-thione (λmax 363 nm; aP 22,500 M−1 cm−1), respectively. Based on the

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favorable spectroscopic properties of 1,3-benzodithiole-2-thione, a general method for the sensitive, specific, and quantitative analysis of other products [from Pessina et al. (5)].

Metabolism of Isothiocyanates in Vivo. Whereas the nonenzymatic reaction of GSH with benzyl-NCS is rapid, this reaction is also catalyzed by rat liver cytosols, presumably promoted by the glutathione transferases present in these preparations (15). The principal metabolic products of isothiocyanates administered p.o. to mammals are the corresponding thiocarbamates. When six male volunteers were fed about 100 μmol of benzyl-NCS, 53.7% of the dose administered was excreted in the urine as N-acetyl-S-(N-benzylthiocarbamoyl)-L-cysteine, a mercapturic acid, in the first 12 h. No other metabolites were detected, and the excretion appeared to be complete in this period (16). Similar results were observed in two men and two women who were fed watercress, a rich source of phenethyl-NCS and its glucosinolates (glucoraphanin), which is hydrolyzed by the myrosinase present in this plant (17). When 30- or 57-g portions of fresh watercress (containing 0.72 mg of glucoraphanin/g) were consumed by each individual, 30 to 67% of the phenethyl-NCS was excreted as N-acetyl-L-cysteine derivative in the urine in 24 h, and the majority of this conjugate was recovered within the initial 8 h (17). This experiment does not provide unequivocal evidence for the hydrolysis of the glucosinolate to isothiocyanate in the body, since no information on the content of free phenethyl-NCS in the watercress was provided. Furthermore, it is not clear whether breakdown of the glucosinolate to isothiocyanate resulted from the myrosinase activity of the watercress or from enzymes present in the test subjects (either in the host tissues or the microbial flora of the gastrointestinal tract). We are not aware of any experimental information on the ability of human subjects to hydrolyze glucosinolates to isothiocyanates.

Rats, mice, guinea pigs, and rabbits likewise converted isothiocyanates efficiently to N-acetyl cysteine conjugates, but other metabolites, including cyclic mercaptopyruvate derivatives (not detected in humans) were also formed (17–20). However, when phenyl-, α-naphthyl-, β-naphthyl, or tert-butyl isothiocyanate were given to rats, no cysteine conjugates were detected (15, 16, 21). The conversion of isothiocyanates to their N-acetyl cysteine derivatives (mercapturic acids) proceeds by the conventional route of conjugation with glutathione presumably promoted by glutathione transferases. The resulting conjugates are then hydrolyzed to the cysteine derivatives and acetylated. Although this pathway has been generally regarded as a detoxication mechanism, recent attention has been directed to the role of GSH and cysteine conjugates (thiocarbamates) as a means of transporting isothiocyanates to peripheral organs where they can undergo cleavage and contribute to toxicity (22, 23) or induce enzymes that protect against toxicity (see below).

Isothiocyanates as Protectors against Chemical Carcinogenesis

The capacity of organic isothiocyanates to block chemical carcinogenesis was recognized 30 years ago (24, 25). Interest in naphthyl-NCS arose from its ability to produce profound proliferative damage in the liver. In long-term feeding experiments α-naphthyl-NCS significantly reduced (in a dose-dependent manner) the formation of liver tumors by 3′-methyl-4-dimethylaminoazobenzene, ethionine, and N-2-fluorenylacetamide in male Wistar rats. Furthermore, animals fed α-naphthyl-NCS did not develop ear duct carcinomas and leukemia, unlike rats receiving the N-2-fluorenylacetamide alone (24, 25). Lacassagne et al. (26) confirmed and extended these findings by demonstrating that not only dietary α-naphthyl-NCS but also β-naphthyl-NCS (which does not cause marked proliferative changes in the liver) blocked hepatic tumor formation in rats fed 4-dimethylaminoazobenzene. Both isothiocyanates produced profound effects on hepatic enzymes that metabolize xenobiotics (27, 28).

These findings laid the groundwork for many subsequent studies on the tumor blocking activities of isothiocyanates, which were administered usually for only short time periods (Table 1).

Mammary Tumors. In the single dose 9,10-dimethyl-1,2-benzanthracene (DMBA)-induced mammary tumor model in female Sprague-Dawley rats, single doses of phenyl-NCS, phenethyl-NCS, and benzyl-NCS markedly reduced the incidence and multiplicity of mammary tumors. In a detailed experiment Wattenberg (29) showed that the timing of administration of the anticarcinogen with respect to the DMBA was very important. Thus, when benzyl-NCS was administered by gavage 2 h prior to the single dose of carcinogen, the average number of rats bearing tumors was reduced from 77% to 8%, and the number of tumors per animal dropped from 1.6 to 0.08. Administration of the isothiocyanate 4 h prior to the carcinogen was somewhat less effective; but if the isothiocyanate was given 24 h before or 4 h after the DMBA, the tumor blocking effect was largely abolished. In other experiments with the same rat tumor model, Wattenberg (30) observed that dietary benzyl isothiocyanate fed for the entire experimental period, beginning 1 week after the DMBA dose, was also highly effective in reducing mammary tumor incidence and multiplicity.

Forestomach and Lung Tumors. Administration of benzyl-NCS (1 mg) to female A/J mice by gavage 15 min prior to each exposure to dimethylnitosamine (20 mg/kg; once weekly for 8 weeks) reduced forestomach tumor incidence and multiplicity but had no effect on pulmonary adenoma formation. In contrast, administration of benzyl-NCS (1 or 2.5 mg, 15 min before carcinogen) reduced, in a dose-dependent manner, both pulmonary adenomas and forestomach tumors evoked by benzo(a)pyrene (3 doses of 2 mg each, given p.o. at 2-week intervals) (31). Benzyl-NCS also suppressed the formation of forestomach tumors in ICR/Ha mice (38).

The effects of isothiocyanates on lung tumors resulting from administration of the potent tobacco-derived nitrosamine carcinogen NNK have been studied intensively and reviewed recently by Chung (39). In male F344 rats, phenethyl-NCS was fed for 1 week prior to and during the course of treatment with NNK (1.76 mg/kg s.c., 3 times weekly for 21 weeks). At the termination of the experiment (104 weeks), the pulmonary tumor incidence (adenomas and carcinomas) was reduced from 80% to 43% by the isothiocyanate treatment, which also inhibited the methylation and pyridyloxobutylation of lung DNA (32).

In shorter term experiments, female A/J mice received 5 μmol of various phenyl-(CH2)1-NCS (n = 0–6) daily by gavage for 4 days.
Two weeks after the final treatment with isothiocyanates, a single dose of NNK (10 μmol by i.p. injection) was administered and pulmonary tumors were quantitated 16 weeks later. Phenyl-NCS and benzyl-NCS were inactive, but phenethyl-NCS (n = 2) and isothiocyanates with n = 3-6 produced marked reductions in lung tumor formation; the inhibition was progressively more pronounced as the methylene chain was lengthened (33, 34). Thus phenylethyl-NCS (5 μmol daily for 4 days) reduced pulmonary tumor multiplicity from 7.9 to essentially zero. DNA methylation, as measured by αP-methylguanine formation, was likewise blocked by these aroylalkyl isothiocyanates, again more markedly as the methylene chain of the isothiocyanates was made longer (34).

If the administration of benzyl-NCS or phenethyl-NCS (1-3 μmol/g of diet) was delayed for 1 week after treatment with NNK and then continued to the end of the experiment at 16 weeks, no effect on pulmonary tumor formation was observed at nontoxic doses of the isothiocyanates (35).

Tumors of the Esophagus. Stoner et al. (36) have reviewed their studies on the inhibition by phenethyl-NCS of esophageal tumor production in the rat by the asymmetrical nitrosamine, N-nitrosobenzylmethyleneimine. The importance of these studies derives from the causative factors for esophageal cancers in some high incidence regions (37). Male F344 rats treated with N-nitrosobenzylmethyleneimine, (0.5 mg/kg s.c. once per week for 15 weeks) developed 100% esophageal tumors at the end of the 25-week assay period, and the tumor multiplicity was negligible (0.1 ± 0.3/animal). At a higher dose of phenethyl-NCS (6 μmol/g of diet) no tumors were observed (37). Phenethyl-NCS treatment blocked the formation of both preneoplastic and neoplastic lesions in the esophagus; even at the lower dose of the isothiocyanate, there was complete inhibition of the appearance of the more advanced (papilloma and carcinoma) lesions (37).

Parallel measurements of the effects of phenethyl-NCS on the metabolism of [3H]nitrosobenzylmethyleneimine in rat esophageal explants demonstrated concentration-dependent inhibition of both metabolism of the carcinogen and methylation of DNA, as indicated by decreased formation of αP-methylguanine and αP-methylguanine.

Tumor-blocking Effects of Glucosinolates. A few experiments have examined the chemoprotective effects of glucosinolates, which are often present in plants in much higher concentrations than their isothiocyanate hydrolysis products. The most clear-cut results have been obtained with the DMBA-induced mammary tumors of the Sprague-Dawley rat. Administration of large single doses of glucobrassicin (indolymethyl glucosinolate) or of glucotropaeolin (benzyl glucosinolate) 4 h prior to the carcinogen substantially reduced both the incidence (from 75% to 25-38%) and the multiplicity (from 1.25 to 0.50-0.69 tumor/rat) of mammary tumors. Administration of these glucosinolates or of glucosinolinate (4-hydroxybenzyl glucosinolate) produced some reduction in the multiplicity, but usually not the incidence, of esophageal tumors and pulmonary adenomas of mice treated with benzyl(α)pyrene (40). The interpretation of these experiments is complicated. It is not known to what extent these glucosinolates are degraded in the rodent bodies or to what extent the glucosinolates or their degradation products are responsible for the tumor-blocking effects. If the glucosinolates are hydrolyzed, it is not known whether endogenous or microbial enzymes are responsible and what products are formed. These issues are especially complicated for glucobrassicin, since there are known degradation products of this glucosinolate (indole-3-acetonitrile, 3,3'-diindolylmethane, and indole-3-carbinol) are anticarcinogens and inducers of cytochrome P-450 (41, 42). A further potentially confounding effect is the conversion of indole-3-carbinol under mildly acid conditions (such as prevail in the stomach) to cyclic derivatives that bind at very low concentrations to the Ah receptor and thereby become very potent inducers of cytochrome P450/1A1 (43).

Mechanisms: Effects of Isothiocyanates on Carcinogen Metabolism

Understanding of the mechanisms of the chemoprotective effects of isothiocyanates is of great importance not only because these substances block the formation of a wide variety of carcinogen-induced tumors in rodents, but also because isothiocyanates and their glucosinolate precursors are widespread in human dietary plants and are consumed in substantial quantities. To what extent these substances contribute to the well-recognized protective effects of vegetables against cancer is unclear (44). The only plausible mechanisms proposed for the anticarcinogenic effects of isothiocyanates implicate modulation of carcinogen metabolism, both depression of activation of carcinogens and acceleration of their disposal. Evidence for these conclusions is based on measurements of: (a) carcinogen-DNA adduct formation and the accompanying nucleotide modifications; (b)
rates of activation of carcinogens and levels of Phase 1 enzymes; and (c) activities of Phase 2 enzymes and levels of GSH.

Reactions of Carcinogens with DNA. There is a striking parallel between the inhibitory effects of various arylalkyl isothiocyanates on lung tumor formation by NNK, the potent carcinogenic nitrosamine of tobacco, and the ability of these isothiocyanates to block O\textsuperscript{\textprime}-methylguanine formation in lungs of rats and mice. The very systematic studies by Chung, Hecht and their colleagues should be consulted for details [Refs. 32, 39, and 45; reviewed by Chung (46)]. These workers and others (46) have also studied the metabolic activation of NNK by lung tissue and microsomes and have concluded that the chemoprotective isothiocyanates act principally on the enzymes involved in the metabolic activation of NNK. By the use of inhibitory antibodies specific for cytochromes P-450, Guo et al. (46) identified cytochromes P450IIIB1 and 2 but not P450IIA1 or P450IIIE1 as important participants in the activation process.

Similar experiments by Stoner et al. (36, 37) have established that the inhibitory effects of phenethyl-NCS on the production of esophageal tumors by N-nitrosobenzylmethylamine in rats also paralleled inhibition of the binding of the carcinogen to DNA and the formation of N\textsuperscript{\textprime}-methylguanine and O\textsuperscript{\textprime}-methylguanine.

Regulation of Phase 1 Enzymes by Isothiocyanates. Administration of isothiocyanates to rodents produced either increases or decreases of microsomal cytochrome P-450 content and the activities of several cytochrome P-450-dependent monooxygenases. The effects appear to depend on experimental conditions: the nature of the isothiocyanate; the treatment regimen; the target tissue examined; and the specific monooxygenase measured.

The most dramatic increases have been reported with indole-3-carbinol and 3,3\textprime;-diindolylmethane and to a lesser degree with indole-3-acetonitrile. These indoles are hydrolytic rearrangement products of glucobrassicin (indolylmethyl glucosinolate). Single i.g. administrations of 10-KK) ¿mol of these indoles to female Sprague-Dawley rats raised the aryl hydrocarbon hydroxylase activities of homogenates of liver and small intestine as much as 25-fold. Clearly these effects cannot be directly attributed to the actions of isothiocyanates (41). As already mentioned,
recent experiments (43) have shown that indole-3-carbinol is readily converted both in vitro and in vivo, under mild acid conditions to polymeric indoles that bind with very high affinity to the Ah receptor, and thereby enhance transcription of cytochrome P450IA1.

Single i.g. doses (100 μmol) of phenethyl-NCS to male F344 rats caused marked increases of liver microsomal pentoxyresorufin O-dealkylase activity (10-fold) and the content of cytochrome P450IIB1 protein (6.6-fold) (47). In contrast, cytochrome P-450 content, ethoxyresorufin O-dealkylase, and erythromycin N-demethylase activities all fell moderately during the first 12–18 h and then recovered by the end of the experiment at 48 h (47). Although these effects appear to be somewhat contradictory, the above-described treatment with phenethyl-NCS depressed the formation of reactive metabolites of NNK by lung (and to a lesser degree by liver) microsomes. The principal effect of phenethyl-NCS appears to be depression of the α-hydroxylation of NNK which is critical for DNA adduct formation. Furthermore, the direct addition of a number of isothiocyanates to microsomes of rat and mouse lungs and rat nasal mucosa potentely depressed the metabolic activation reactions of NNK. Thus, 50% inhibition was achieved by phenylethyl-NCS at 120–200 nM, by phenylbutyl-NCS at 30–75 nM, and by phenylethyl-NCS at 15–90 nM depending on the specific metabolites. The Ki values for the inhibition of NNK metabolism by phenylethyl-NCS were very low (10.9–16.5 nM) for various reactions (57).

Straightforward results were obtained in experiments in which 100–300 μmol daily i.g. doses of allyl-NCS were given to male outbred rats for 3 days. The hepatic aminopyrine α-demethylase, p-nitroanisole O-demethylase, and aniline p-hydroxylation activities were all decreased in a dose-dependent manner as low as 31–46% of control values (48).

Although both α- and β-naphthyl-NCS block neoplasia in the liver (see above), dietary administration of these compounds to male F344 rats for 2–4 weeks had opposite effects on cytochrome P-450 content and monooxygenase activities of liver microsomes. Whereas the α-naphthyl-NCS depressed total P-450 content, as well as ethoxyresorufin O-deethylation and benzphetamine O-demethylase activities, the β isomer had the opposite effects (27, 28). For instance, dietary β-naphthyl-NCS (5.4 μmol/g diet for 28 days) raised microsomal cytochrome P-450 content, ethoxyresorufin O-deethylation, and benzphetamine N-demethylase 1.6-, 1.74-, and 2.2-fold, respectively (28). In Hepa 1c1c7 murine hepatoma cells in culture, benzyl-NCS had no effect on aryl hydrocarbon hydroxylase activity (8).

Although the effects of isothiocyanates on Phase 1 enzymes appear to vary greatly with experimental conditions and the activity measured, the conclusion that these compounds depress the activation metabolism of two carcinogens, NNK and N-nitrosobenzylmethyamine, appears to be firmly established.

**Regulation of Phase 2 Enzymes and Glutathione Levels by Isothiocyanates**

In contrast to the complex effects of isothiocyanates on Phase 1 enzymes, the effect of these agents on Phase 2 enzymes of rodent tissues is quite straightforward. Table 2 summarizes the changes in specific activities of GST and QR in the cytosols of several organs of mice and rats treated with benzyl-NCS, phenethyl-NCS, allyl-NCS, α-naphthyl-NCS, β-naphthyl-NCS, or sulforaphane [1-isothiocyanato-(4R)-methylsulfinylbutane]. The compounds were given either in the diet (3–34 μmol/g diet) for 5–28 days or by i.g. administration (5–100 μmol in single or several daily doses). The organs examined included: liver; esophagus; forestomach; glandular stomach; small bowel; colon; lung; kidney; and bladder. Inductions of GST and QR, expressed as ratios of specific activities of tissues obtained from treated and control animals varied from 1.2 to 9.4 and were mostly in the 2–4-fold elevation range. Phase 2 enzyme induction, as represented by elevations of GST and QR in various tissues, appears to be a constant property of a variety of isothiocyanates. Benzyl-NCS also raised GSH levels in esophagus and small bowel (but not forestomach) of mice by 63–75% (Table 3; Ref. 38).

In common with many chemically unrelated chemoprotective compounds, the administration of isothiocyanates to rodents evokes a generalized "electrophile counterattack" response, characterized by the induction of Phase 2 enzymes and increases in tissue GSH levels (54, 55).

Much has been learned about the chemical structure and mechanism of induction of chemoprotective compounds by the measurement of QR activity in Hepa 1c1c7 murine hepatoma cells in culture (7), and especially in cells grown in 96-well microtiter plates (56). Elevation of QR in this cell line accurately predicts induction of both QR and GST in animal tissues. This system played a critical role in the identification of sulforaphane as the major Phase 2 enzyme inducer in SAGA broccoli (10). Extensive information is now available on the inducer potency of a wide variety of isothiocyanates in Hepa cells. Some illustrative inducer concentrations required in the assay system to double the specific activity of QR in Hepa 1c1c7 murine hepatoma cells are given in Table 4.

In an effort to understand the basis for the high inducer potency of sulforaphane, a large number of bifunctional isothiocyanates were recently synthesized and evaluated as inducers (58). It was found that among these analogues, the methylsulfinyl group (CH₃SO—) of sulforaphane could be replaced by either CH₃CO— or CH₂SO₂— groups without significant effect on inducer potency. For optimal potency, these functional groups had to be separated from the —NCS function by 3 or 4 carbon atoms. In some of the analogues, the link separating the two functions was flexible whereas in others it was relatively rigid. Certain bifunctional norbornyl-NCS analogues were potent inducers of QR in murine hepatoma cells and inducers of QR and GST in mouse tissues. Since there is much persuasive evidence that induction of Phase 2 enzymes plays a major role in the chemoprotective effects of many different classes of compounds (9), it appears very likely that isothiocyanates also exert at least a part of their protective actions through this mechanism. Isothiocyanates are potent electrophiles like most other inducers of Phase 2 enzymes (7, 54, 55). Isothiocyanates resemble other monofunctional enzyme inducers in that they stimulate transcription of Phase 2 enzymes via a common AP-1-like enhancer element present in the upstream regulatory regions of certain GST and QR genes (54, 55).

**References**


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