Chemoprotection against cancer by isothiocyanates and glucosinolates

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Introduction

Organic isothiocyanates (R—N=C=S) and glucosinolates, their biosynthetic precursors in plants, are attracting increasing attention as chemical and dietary protectors against cancer. More than 20 natural and synthetic isothiocyanates and several glucosinolates have been shown to block chemical carcinogenesis in animal models (see reviews in [1,2]); moreover, these substances are widely and often abundantly distributed in edible plants. The human consumption of isothiocyanates and glucosinolates is estimated at milligram quantities daily [3,4]. Nevertheless, it remains unclear to what extent these phytochemicals contribute to the well-recognized observations that individuals who consume large amounts of vegetables have lower risks of developing cancer (see review [5]). More than 100 isothiocyanates and glucosinolates have been isolated from plants, many of which belong to the family Cruciferae and more specifically to the genus Brassica (e.g. Brassica oleracea sp.: cabbage, cauliflower, Brussels sprouts, broccoli, kale) and the genus Raphanus (radishes and daikons) [3]. In addition, a large number of isothiocyanates have been synthesized for cancer chemoprotection studies [6,7].

Isothiocyanates are biosynthesized and stored in plants as relatively stable precursors known as glucosinolates (β-thioglucoside N-hydroxysulphates) [3]. The same plants also produce myrosinase (thioglucoside glucohydrolase, EC 3.2.3.1) which normally is structurally segregated from its glucosinolate substrates, but is liberated when plant cells are damaged (by food preparation or eating) and promotes the hydrolysis of glucosinolates to isothiocyanates (as well as other products), hydrogen sulphate and glucose:

\[
\text{S—C}_6\text{H}_{11}\text{O}_3 \xrightarrow{\text{Thioglucosidase}} \text{R—N=C=S} + \text{H}_2\text{O} + \text{SO}_3^- + \text{C}_6\text{H}_{12}\text{O}_6 + \text{HSO}_\text{3}\]

Chemistry and metabolism

Unlike glucosinolates, which are relatively stable and unreactive, isothiocyanates (R—N=C=S) contain a highly electrophilic central carbon atom that reacts rapidly under mild conditions with oxygen-, sulphur- or nitrogen-centred nucleophiles to give rise to thiocarbamates, dithiocarbamates or thiourea derivatives respectively [13]. Conjugates of isothiocyanates with GSH are especially important in vivo since they lead to the formation of dithiocarbamates, dithiocarbamates or thiourea derivatives respectively [1]. Conjugates of isothiocyanates with GSH are especially important in vivo since they lead to the formation of dithiocarbamates which are the major products of isothiocyanate metabolism. Although such conjugations occur non-enzymatically, they are greatly accelerated by glutathione transferases (GSTs). These conjugations are catalysed by all cloned human GSTs tested, including GST A1–1, P1–1, M2–2 and M4–4 [8,9]. Although the conjugation with GSH is reversible and cleavage of the conjugates is also accelerated by GSTs, our studies indicate that the equilibria are quickly established and that the conjugation reaction is strongly favoured. The conjugates are rather poor sub-
Bioadive Components of Food

strates of GSTs, and their rates of cleavage are at least 1000-fold slower than their rates of formation under similar conditions [8,10]. It is therefore unlikely that significant cleavage of dithiocarbamates occurs in the presence of the normally high GSH concentrations that prevail in most tissues.

Studies on the fate of several ingested isothiocyanates in humans have indicated that a major route of metabolism is their conversion into N-acetylcysteine derivatives (mercapturic acids). When benzyl-NCS or phenylethyl-NCS (46–94 pmol) was ingested, approximately 50% of the administered doses of these compounds were excreted in the urine as the N-acetylcycteine conjugates in less than 12 h [4,11]. Similar results were obtained in rats [12,13]. The conversion of isothiocyanates into their N-acetylcysteine derivatives proceeds by the conventional route of initial conjugation with GSH promoted by GSTs, followed by hydrolysis of the resulting conjugates to the cysteine derivatives and final N-acetylation.

Anticarcinogenic activity of organic isothiocyanates

The capacity of organic isothiocyanates to block chemical carcinogenesis was first recognized more than 30 years ago with α-naphthyl-NCS (see [1]). Since then, about 20 natural and synthetic isothiocyanates have been shown to inhibit chemical carcinogenesis. The anticarcinogenic activities of isothiocyanates have been demonstrated in rodents (mice and rats) with a wide variety of chemical carcinogens (including polycyclic aromatic hydrocarbons, azo dyes, ethionine, fluorenylacetamide and several nitrosamines). Protection is afforded in a variety of target organs: lung, liver, mammary gland, oesophagus, small intestine, colon and bladder [1,2]. The isothiocyanates and glucosinolates known to be effective as chemoprotectors in various animal models are listed in Table 1. These isothiocyanates vary considerably in structure and show some degree of organ- and carcinogen-specificity. For instance, phenethyl-NCS, which is very potent in blocking NNK lung carcinogenesis in A/J mice, did not prevent NNK-induced skin tumour formation in the same strains of mice [6]. Phenethyl-NCS even enhanced azoxymethane-induced colon tumorigenesis and N-nitrosomethylbenzylamine (N MBA)-induced oesophageal carcinogenesis in rats [14,15]. Benzyl-NCS and several other isothiocyanates, in contrast, block tumorigenesis by multiple carcinogens in a number of tissues of both rats and mice (see Table 1). Most isothiocyanates have shown chemoprotective activity in protocols involving administration of the isothiocyanate either before or during exposure to the carcinogen. Only benzyl-NCS inhibited DMBA-induced mammary tumour development when administered to rats after a single dose of DMBA [16]. Several glucosinolates have also been tested [17]. Glucobrassicin inhibited benzo[a]pyrene-induced pulmonary adenomas in ICR/Ha mice. Glucobrassicin and glucotropaeolin inhibited the development of DMBA-induced mammary tumours in Sprague–Dawley rats. Glucobrassicin, glucotropaeolin and glucosinalbin inhibited

<table>
<thead>
<tr>
<th>Carcinogens</th>
<th>Protective isothiocyanates</th>
<th>Protective glucosinolates</th>
<th>Tumour target organs</th>
</tr>
</thead>
<tbody>
<tr>
<td>3′-Methyl-4-dimethylaminoazobenzene</td>
<td>α-Naphthyl-NCS, β-naphthyl-NCS</td>
<td>Indolylmethyl glucosinolate (glucobrassicin)</td>
<td>Rat: liver, lung, mammary gland, bladder, small intestine/colon, oesophagus</td>
</tr>
<tr>
<td>4-Dimethylaminoazobenzene</td>
<td>Phenyl-[CH₂]ₙ-NCS, where n = 0, 1, 2, 3, 4, 5, 6, 8, 10</td>
<td>Benzyl glucosinolate (glucotropaeolin)</td>
<td>Mouse: lung, forestomach</td>
</tr>
<tr>
<td>N-2-Fluorenylacetamide, acetylaminofluorene</td>
<td>PhCH(Ph)CH₂-NCS; PhCH₂CH(Ph)-NCS</td>
<td>4-Hydroxybenzyl glucosinolate (glucosinalbin)</td>
<td></td>
</tr>
<tr>
<td>7, 12-Dimethylbenzannelanthracene (DMBA)</td>
<td>CH₃(CH=CH₂)-NCS, where n = 5, 11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>CH₃(CH=CH₂)CH(CH₃)-NCS</td>
<td>Sulphoraphane, CH₃S(O)[CH₂₃⁺]₂-NCS</td>
<td></td>
</tr>
<tr>
<td>Methylazoxymethanol acetate</td>
<td>CH₃₂CH₃CH(CH₃)-NCS</td>
<td>2-Acetylornorboryl-NCS (3 isomers)</td>
<td></td>
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<tr>
<td>N-Nitrosodiethylamine</td>
<td>Sulphoraphane, CH₃S(O)[CH₂₃⁺]₂-NCS</td>
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<tr>
<td>4-(Methylnitrosamino)- 1-(3-pyridyl)-1-butaneone (NNK)</td>
<td>Sulphoraphane, CH₃S(O)[CH₂₃⁺]₂-NCS</td>
<td></td>
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<tr>
<td>N-Nitrosobenzylmethylamine (NBMA)</td>
<td>Sulphoraphane, CH₃S(O)[CH₂₃⁺]₂-NCS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Butyl-N-(4-hydroxybutyl)nitrosamine</td>
<td>Sulphoraphane, CH₃S(O)[CH₂₃⁺]₂-NCS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: Zhang and Talalay [1]; Hecht [2]. Only compounds for which protection has been demonstrated are listed.

Table 1

Protection by a variety of isothiocyanates and glucosinolates against chemical carcinogenesis in rat and mouse organs
benzo[a]pyrene-induced pulmonary adenomas in mice [17]. It is not clear whether the glucosinolates themselves or their hydrolysis products are the active chemical species responsible for the protective effects. Glucobrassicin has been shown to be hydrolysed to indole-3-acetonitrile, indole-3-carbinol and 3,3′-di-indolymethane, all of which are good inducers of aryl hydrocarbon hydroxylase (cytochrome P-450IA1) activity [18]. Furthermore, indole-3-carbinol is rapidly converted under slightly acidic conditions (such as prevail in the stomach) into polymeric species that bind to the aryl hydrocarbon receptor with extremely high affinity and induce cytochrome P-450IA1 [19].

**Mechanisms of chemoprotective effects of isothiocyanates**

Elucidation of the mechanisms underlying the chemoprotective effects of isothiocyanates (and of their glucosinolate precursors) is of critical importance, not only because these agents offer substantial promise as dietary chemoprotectors in humans, but also because better understanding of their protective mechanism may lead to the design of more effective protectors. Current mechanisms proposed for the anticarcinogenic effects of isothiocyanates involve modulation of carcinogen metabolism: both depression of activation of carcinogens by Phase 1 enzymes and acceleration of their disposal by Phase 2 enzymes. Phase 1 enzymes (e.g. cytochromes P-450) functionalize xenobiotics by oxidation or reduction, and their primary role is to convert xenobiotics into substrates for Phase 2 enzymes. The latter conjugate functionalized products with endogenous ligands (e.g. GSH or glucuronic acid) or destroy reactive centres by other reactions [e.g. hydrolysis of epoxides by epoxide hydrolase or reduction of quinones by quinone reductase (QR)]. After such reactions, electrophilic carcinogens are nearly always less reactive and can be excreted more easily. On rare occasions, Phase 1 enzymes play an important role in activating procarcinogens to highly reactive ultimate carcinogens.

Recently, however, benzyl-NCS and phenylethyl-NCS were also shown to delay cell cycle progression of HeLa cells, resulting in inhibition of cell growth [20], and this may be another mechanism contributing to the chemoprotective activities of isothiocyanates.

**Depression of activation of carcinogens**

The most detailed and systematic studies of carcinogen activation and its inhibition by isothiocyanates have been reported for two nitroamine carcinogens: NNK, the most potent tobacco carcinogenic nitrosamine in rodent lung tumorigenesis (and presumably in humans), and NBMA, the most potent oesophageal carcinogen in rodents. In studies reviewed elsewhere [1,2,4,21], it has been convincingly established that various natural and synthetic isothiocyanates depress the activational metabolism of the carcinogens, NNK and NBMA, by inhibiting the cytochromes P-450 involved in the activation process. There is also a striking parallel between the inhibitory effects of various arylalkyl isothiocyanates on lung tumour formation by NNK and the ability of these isothiocyanates to block O⁺-methylguanine formation in lungs of rats and mice [22-24]. The largest protective effects on tumour inhibition were observed when the isothiocyanate was given before or during (but not after) NNK treatment [25,26]. These findings are consistent with the conclusion that in vitro certain isothiocyanates are direct and very potent inhibitors (both competitive and irreversible, depending on conditions) of the cytochromes P-450 involved in NNK activation. In a detailed correlative comparison of the arylalkyl isothiocyanates and some alkyl isothiocyanates, Jiao and co-workers [27] found that: (a) increased alkyl chain length (Ph-[CH₂]ₙ-NCS, n = 0-6, 8, 10) enhances the inhibitory activity of arylalkyl isothiocyanates toward NNK lung tumorigenesis; (b) the phenyl moiety is not essential for the inhibitory activity, since a long-chain alkyl isothiocyanate (e.g. CH₃-[CH₂]₁₁-NCS) also exhibits strong inhibitory effects in the same model; and (c) the inhibitory potency of isothiocyanates is positively correlated with lipophilicity [i.e. log P (partition coefficients between octanol and aqueous phases)] and is inversely correlated with the rate constants of reaction with glutathione [6,27]. In these experiments, single or four daily doses of the isothiocyanate were administered 2 h before the single dose of NNK. However, the same arylalkyl isothiocyanates behaved differently in their inhibition of NMBA-induced oesophageal tumorigenesis in F344 rats. The potency of the inhibitory effects of these isothiocyanates (Ph-[CH₂]ₙ-NCS, n = 1-4) on NMBA-induced oesophageal tumorigenesis and DNA methylation followed the order n = 3 > 2 > 4 > 1 [28]. Moreover, when the experiment
was terminated 4 weeks early, it was found that phenylhexyl-NCS (Ph-[CH₂]₆-NCS) actually enhanced NMBA carcinogenesis in rat oesophagus [15]. In these experiments, the isothiocyanates were administered continuously, starting 2 weeks before NMBA, which was given once weekly for 15 weeks. Thus the ability of isothiocyanates to inhibit or enhance tumorigenesis depends on the structure of the isothiocyanates, the animal species and target tissues, and the specific carcinogen employed. Studies by Yang and co-workers [29] also showed that isothiocyanates are both inhibitors of some Phase 1 enzymes and inducers of other Phase 1 enzymes.

It is therefore of interest that sulphoraphane \( \{\text{CH}_3\text{S(O)}[\text{CH}_2]_{n}\text{-NCS}\} \), which is the very potent Phase 2 enzyme inducer isolated from broccoli [30], has been recently shown to inhibit at least one cytochrome \( P-450 \) (CYP2E1) involved in the activation of certain carcinogens, and also to depress the mutagenic capacity of \( N \)-nitrosodimethylamine \textit{in vivo} [31].

### Induction of Phase 2 enzymes: acceleration of carcinogen disposal

As described above, reactions of electrophilic carcinogens catalysed by Phase 2 enzymes generally lead to decreased reactivity and increased excretion of carcinogens. Phase 2 enzymes (e.g. GSTs, UDP-glucuronosyltransferases and sulphotransferases) promote conjugation of electrophilic carcinogens with endogenous ligands, or deposit their reactive centres by other reactions (e.g. epoxide hydrolase and QR), thus facilitating their excretion from the body. When exposed to low concentrations of electrophiles, mammalian cells undergo a generalized 'electrophile counter-attack' response, characterized by the induction of Phase 2 enzymes and increases in tissue GSH levels [32]. Phase 2 enzyme induction, as represented by increases in QR and GST activity in various rodent tissues, is a constant property of a variety of isothiocyanates. Aromatic isothiocyanates \( (\text{C}_n\text{H}_2-[\text{CH}_2]_n\text{-NCS, } n = 1, 2, 4, 6), \) \( \alpha \)-or \( \beta \)-naphthyl-NCS, allyl-NCS, \( \text{CH}_3\text{S}[\text{O}_2]_2-[\text{CH}_2]_n\text{-NCS (n = 0, erucin; n = 1, sulphoraphane; n = 2, erysolin) and exo-2-acetyl-exo-6-isothiocyanato-norbornane are inducers of QR and GST in several organs of mice and rats, including liver, small bowel, colon, kidney, stomach, lung and nasal mucosa. The compounds were given either in the diet (3-4 \text{ mmol/g of diet}) for 5-28 days or by intragastric administration (5-100 \text{ mmol in single or several daily doses}), and the specific activities of GST and QR in the cytosols of these organs were increased from 1.2- to 9.4-fold over those of control animals (see [1]). The co-ordinate nature of induction of Phase 2 enzymes has been studied in detail in Wistar rats with benzyl-NCS. Benzyl-NCS (0.5% (w/w) in the diet for 2 weeks) increased liver and small intestinal GST, QR and UDP-glucuronosyltransferase activities by 1.73-11-fold [33]. Benzyl-NCS also raised GSH levels in oesophagus and small bowel of ICR/Ha mice by 63-75% [34]. Thus, in common with many chemically unrelated inducers, administration of isothiocyanates to rodents evokes the 'electrophile counter-attack' response. Molecular studies have shown that isothiocyanates can induce Phase 2 enzymes by stimulating transcription of these genes via a common antioxidant/electrophile (ARE/EpRE) enhancer element present in the upstream regions of several Phase 2 enzyme genes [35].

### Conclusions

Organic isothiocyanates and glucosinolates block the development of tumours produced by a variety of carcinogens in various organs of rodents. These anticarcinogenic effects of isothiocyanates appear to be mediated by tandem, and possibly synergizing, mechanisms: inhibition of carcinogen activation by cytochromes \( P-450 \) (Phase 1 enzymes) and enhanced detoxication of activated carcinogens by induction of Phase 2 enzymes. The extent to which each mechanism contributes to the anticarcinogenic activities of isothiocyanates probably depends on the carcinogen, the tumour target organ and the chemical nature of the isothiocyanate. Structure–activity studies with natural and synthetic isothiocyanates have revealed that, whereas hydrophobicity and low reactivity of isothiocyanates with GSH correlate with greater potency of inhibition of lung tumour formation by NNK in mice, this correlation does not extend to the blocking of oesophageal tumours produced by NBMA in rats. The rational design of isothiocyanates for chemoprotection will therefore require very detailed biological evaluation of each compound in a number of biological systems: (a) several chemically induced tumour systems in rodents; (b) inhibition of the activation of the requisite carcinogens by the appropriate cytochromes \( P-450 \); (c) measurement of inducer potency for Phase 2 enzymes in cells and various rodent tissues; and (d) measurement of adduct forma-
ation of carcinogens with DNA. The results of such studies may suggest the use of combinations of isothiocyanates – potent inhibitors of cytochromes P-450 together with potent inducers of Phase 2 enzymes – in order to achieve maximum chemoprotection.

There is therefore much promise in chemoprotection studies with isothiocyanates and glucosinolates because these substances are already present in the human diet and they have such a wide spectrum of tumour-blocking activities.


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