Chapter 8

Summary

Cruciferous vegetables

Cruciferous vegetables belong to the botanical family Brassicaceae. The greatest consumption of cruciferous vegetables has been reported to be that of adults in China, who may eat more than 100 g per day. Other Asian populations also have a relatively large consumption of cruciferous vegetables, ranging from 40 to 80 g per day. The average cruciferous vegetable intake in North America is reported to be 25–30 g per day. The consumption in European countries varies substantially, intake in some countries in central and northern Europe being more than 30 g per day and that in some southern European countries being less than 15 g per day. This pattern differs from that for the consumption of all vegetables, which shows a decreasing gradient from south to north in Europe. Relatively small amounts of cruciferous vegetables, 15 g per day or less, are reported to be eaten in South Africa and some countries in South America, and less than 20 g per day in India.

Overall, cruciferous vegetable intake would appear to account for 10–15% of total vegetable intake, ranging from almost 25% in countries with high consumption to only 5% in countries with low consumption. In 2000, cruciferous vegetables represented 25% of all vegetables produced in eastern Europe, about 10% of those produced in Australia, eastern and South-East Asia and western Europe, and less than 10% of those produced in the rest of the world. Between 1962 and 2002, there was a decreasing trend in the production of cruciferous vegetables in Australia, New Zealand and western Europe.

Glucosinolates, isothiocyanates and indoles

Isothiocyanate and indole compounds are derived from consumption of cruciferous vegetables and manufactured products, including condiments, sauerkraut and *kimchi*. Although these two groups of compounds appear to be structurally unrelated, they are both derived from degradation of glucosinolates, the characteristic sulfur-containing glycosides found in cruciferous vegetables. After tissue disruption, glucosinolates are degraded by the action of β-thioglucosidases, commonly known as myrosinases.

Although over 90 different naturally occurring isothiocyanates (ITCs) have been described, people commonly consume only about six: 4-methylsulfinylbutyl- (sulforaphane), 2-propenyl- (allyl), 3-butenyl-, 4-pentenyl-, 3-methylsulfinylpropyl- (iberin) and phenethyl-ITC. Sulforaphane is derived predominantly from broccoli but can also be obtained from rocket (*Eruca sativa*). 2-Propenyl- and 3-butenyl-ITC and iberin are derived from consumption of cultivars of *B. oleracea*. 3-Butenyl- and 4-pentenyl-ITC are derived from consumption of leafy *B. rapa* crops, particularly Chinese cabbage. 2-Propenyl-ITC is also derived from leafy mustard vegetables. Phenethyl-ITC is derived from watercress and to a lesser extent from root crops such as turnips and rutabaga. Benzyl-ITC is relatively rare in the diet, being obtained from cress (*Lepidium*) species. 6-Methylsulfinylhexyl-ITC is derived from *wasabi*.

Six indole glucosinolates have been identified in cruciferous vegetables. Of these, only two are found frequently in the diet: 3-indolylmethyl glucosinolate (glucobrassicin) and 1-methoxy-3-indolylmethyl glucosinolate (neoglucobrassicin) These are found in most cruciferous vegetables. Indole glucosinolates degrade mainly to indole-3-carbinol, which condenses to form 3-3′-diindolylmethane, or to 1-methoxy-indole-3-carbinol. Further condensation reactions may occur, particularly in the acid conditions of the stomach, to produce a series of oligomeric products. Indole glucosinolates can also react with ascorbic acid to form ascorbigen.

Four factors determine exposure of the human gastrointestinal tract to isothiocyanates and indoles: (1) the genetics of glucosinolate biosynthesis within the crop plant, which determines the chemical structure of the degradation products and partially determines the overall amount; (2) abiotic and biotic environmental factors, which can influence the overall amounts of glucosinolates and degradation products produced by the plant; (3) post-harvest storage, processing and cooking; and (4) the myrosinase-like activity of the intestinal microbial flora.

Metabolism, kinetics and genetic variation

Humans

Ingested isothiocyanates are metabolized principally through the mercapturic acid pathway and excreted in urine as dithiocarbamates, mainly in the form of *N*-acetylcysteine conjugates. The initial reaction with glutathione (GSH) may be either spontaneous or may be catalysed by GSH transferases (GSTs). The role of GST polymorphisms in exposure of tissues to isothiocyanates and excretion of isothiocyanates remains unresolved. Analytical methods, especially the cyclocondensation assay, have enabled quantification of isothiocyanates in cruciferous vegetables and of isothiocyanates and their dithiocarbamate metabolites (isothiocyanate equivalents) in human fluids, including blood and urine. The total quantity of urinary isothiocyanate equivalents has been shown to be a reliable marker of human dietary intake of these compounds and to correlate positively with the consumption of cruciferous vegetables.

After intake of indole-3-carbinol, several condensation products are formed, either in the acid environment of the stomach or in the near-neutral environment of the large intestine. 3,3′-Diindolylmethane can be detected in plasma and urine of volunteers given indole-3-carbinol.

Experimental systems

Only limited information is available on the metabolic fate of isothiocyanate compounds, indoles and the nitrile forms in animals fed cruciferous vegetables. The fate of several purified isothiocyanates, including benzyl-, phenethyl- and allyl-ITCs and sulforaphane, has been investigated in rodents. The main route of metabolism of isothiocyanates involves conjugation with GSH and excretion via the mercapturic acid pathway, but minor pathways, such as hydrolysis, oxidation–reduction, ring hydroxylation and alkyl-chain degradation, may be used, depending on the structure of the compound. Analysis of urinary metabolites has shown that there are species differences in the metabolism of isothiocyanates. Studies with radiolabelled isothiocyanates show that these compounds are readily absorbed into blood and tissues and are eliminated almost completely within 24–48 h of oral administration.

Little information is available on the metabolism and distribution of indoles in animals fed cruciferous vegetables. The fate of purified indole-3-carbinol has been examined in rats and trout, whereas the fate of ascorbigen has been studied only in mice. Purified indole glucosinolates have also been studied. The primary indole-3-carbinol derivatives in liver, intestine and serum are 3,3′-diindolylmethane, indolo[3,2-*b*]carbazole and other oligomeric acid condensation products, at ratios and amounts that vary with species and tissue. It is these compounds, not parent indole-3-carbinol, that are believed to be responsible for the organ-specific changes in carcinogen-, endogenous estrogen- and drug-metabolizing enzymes that can occur after ingestion of indole-3-carbinol.

Cruciferous vegetables, isothiocyanates and indoles modulate phase I and phase II enzymes in animals. The modulating effects of isothiocyanates are complex, as they depend on many factors, including species, tissue, treatment protocol, dose and enzyme specificity. Isothiocyanates usually inhibit cytochrome P450 enzymes, whereas indoles can have either inhibitory or stimulatory effects. The mechanism of inhibition of cytochrome P450 enzymes by isothiocyanates and indoles may involve both competitive and non-competitive binding of target enzymes. Studies of structure–activity relationships have shown that aromatic isothiocyanate compounds with longer alkyl chains or greater lipophilicity have enhanced inhibitory action against these enzymes. These studies have resulted in identification of some isothiocyanates that are remarkably powerful inhibitors of cytochrome P450 enzymes.

Cancer preventive effects

Humans

Studies were considered in this evaluation only if the reports provided estimates of risk along with statistical confidence intervals for estimated consumption of all cruciferous vegetables or for specific cruciferous vegetables. The summaries below include only those studies that met these criteria (see Figures 17–24, pp. 94–97).

Most of the epidemiological studies relating cruciferous vegetable intake to cancer risk also included measures of the intake of many other vegetables. Few studies found inverse associations with cancer that were stronger for cruciferous vegetables than for all vegetables. In interpreting the evidence, therefore, it should be recognized that the evidence often was based on analyses of subgroups by vegetable type, and that confounding of cruciferous vegetable intake by total vegetable intake was possible. In only a few studies (especially those of thyroid cancer) was an association with cruciferous vegetable intake the primary hypothesis. When cohort studies were available, they were given more weight, because of the potential biases in sampling or recall that are associated with case–control studies.

Oral cavity and pharynx

One cohort study and three case–control studies gave inconsistent findings.

Oesophagus
Only one case–control study was available, in which no association with Chinese cabbage consumption was found.

Stomach
Three cohort and nine case–control studies were available; most showed inverse associations with cruciferous vegetable intake. Overall, there was a non-significant inverse association in the cohort studies and a statistically significant inverse association in the case–control studies.

Colon and rectum
Six cohort and five case–control studies of colorectal cancer were available. Overall, there was no association in the cohort studies, with as many positive as inverse associations. Four of the case–control studies showed inverse associations with cruciferous vegetable intake, and one case–control study on isothiocyanate intake showed a non-significant inverse association. Overall, there was a statistically significant association. In addition, one cohort and four case–control studies of colorectal adenomas were available. The cohort study showed no association; one of the case–control studies showed statistically significant inverse associations with cruciferous vegetable intake, but one showed a non-significant positive association in women.

Pancreas
One cohort and two case–control studies were available. The cohort study showed no association. Both case–control studies showed inverse associations with cruciferous vegetable intake.

Larynx
Two case–control studies were available; one showed a positive association with cruciferous vegetable intake in women.

Lung
Five cohort and six case–control studies were available; most showed inverse associations with cruciferous vegetable intake, and the results were statistically significant in three cohort studies. In a pooled analysis of cohort studies, however, no association with broccoli or cabbage consumption was found. One cohort and two case–control studies showed statistically significant inverse associations between dietary or urinary isothiocyanate concentration and lung cancer. Overall, there was a statistically significant inverse association in both the cohort and the case–control studies.

Breast
One cohort and eight case–control studies were available; most, as well as a pooled analysis of seven cohort studies, showed no association with cruciferous vegetable intake. In one large case–control study, however, a statistically significant inverse association was found. In addition, a statistically significant inverse association with urinary isothiocyanate concentration was observed in one case–control study. Overall, the cohort study showed an association close to null, while the case–control studies showed a statistically significant inverse association.

Cervix
In two case–control studies, inverse associations with cruciferous vegetable intake were reported, one being statistically significant.

Endometrium
Three case–control studies were available; one showed a statistically significant inverse association with cruciferous vegetable intake.

Ovary
Three case–control studies were available; one showed a statistically significant inverse association with cruciferous vegetable intake.

Prostate
Three cohort and four case–control studies were available. There was no association observed in the cohort studies. In two of the case–control studies, statistically significant inverse associations with cruciferous vegetable intake were reported. In one of these, the association was stronger for advanced disease.

Urinary bladder
Two cohort and one case–control studies were available. In one cohort study, a statistically significant inverse association with cruciferous vegetable intake was reported, and a non-significant inverse association was found in the case–control study.

Kidney
Five case–control studies were available; in two, a statistically significant inverse association with cruciferous vegetable intake was reported, but one was limited to a subgroup. In a pooled analysis of four case–control studies, a statistically significant inverse association was found only among non-smokers.

Brain
One case–control study of childhood brain cancer was available; it showed no inverse association with the cruciferous vegetable intake of the mother during pregnancy.

Thyroid
In a collaborative re-analysis of 11 case–control studies, no association was found. One case–control study showed a statistically significant inverse association with cruciferous vegetable intake, and another showed a non-significant inverse association in females only. In another, an inverse association was found for Brussels

sprouts, but a positive association was found with cabbage and cauliflower intake.

Non-Hodgkin lymphoma

Two cohort studies were available; in one, a statistically significant inverse association with cruciferous vegetable intake was reported. No association was found in the other.

Interaction with glutathione *S*-transferase gene polymorphism

Eight studies were conducted to investigate the effect of GST gene polymorphism on the association between intake of cruciferous vegetables or isothiocyanates and cancer. Four of these were conducted in Chinese populations, and in two of the studies a urinary biomarker of isothiocyanate was used. In four studies on lung cancer (one cohort, three case–control) and a single case–control study on breast cancer, a more consistent inverse association between isothiocyanate or cruciferous vegetable intake and cancer risk was found for individuals null for the *GSTM1* or *GSTT1* genotype. The difference in odds ratios between subgroups defined by GST genotype was statistically significant in some, but not all, studies. This effect of GST gene polymorphism was observed in both smokers and non-smokers and was more consistent in studies in which urinary isothiocyanate concentration was used as the exposure variable than in those in which self-reported food frequency was used to assess dietary intake.

Intermediate effect biomarkers

In humans, intake of cruciferous vegetables, isothiocyanates and indoles has been shown to modulate biotransformation enzymes, which can lead to enhancement of both bioactivation and detoxification of various carcinogens and alterations in steroid hormone metabolism. Studies involving intermediate effect biomarkers could not, however, be interpreted in relation to cancer risk. Consequently, intermediate effects were not used for evaluating the chemopreventive effects of cruciferous vegetables, isothiocyanates or indoles in humans.

Experimental animals

Cruciferous vegetables

In experimental animals treated with carcinogens, dietary intake of cruciferous vegetables in amounts similar to those eaten by human populations have been shown to inhibit neoplastic and preneoplastic changes, especially in the colon, mammary gland and liver. These inhibitory effects were most apparent when the vegetables were given simultaneously with the carcinogen or throughout the experiment. Mustard seed was shown to inhibit skin carcinogenesis as well as transplacental or translactational carcinogenesis in mouse models; wasabi was shown to inhibit stomach tumorigenesis; and dietary cabbage and collards both decreased lung metastasis from injected mammary cancer cells.

Glucosinolates

Sinigrin (2-propenyl glucosinolate), which yields allyl-ITC, reduced the total number of colonic aberrant crypt foci induced by dimethylhydrazine and the incidence of hepatic tumours induced by *N*-nitrosodiethylamine in rats.

Isothiocyanates

Both naturally occurring and synthetic isothiocyanates have been evaluated for their preventive effects against carcinogen-induced tumours in rats, mice and hamsters. The isothiocyanates were administered to rodents by various routes, usually at more than one dose and in protocols to determine their efficacy as either 'blocking' (anti-initiation) or 'suppressing' (anti-promotion or progression) agents.

Phenethyl-ITC potently inhibited tumour development in the oesophagus when administered in the diet before, during and after treatment with *N*-nitrosomethylbenzylamine (NMBA), although it did not inhibit tumour development in rats pre-initiated with this carcinogen. Dietary phenethyl-ITC inhibited the total number of aberrant crypt foci in rat colon when administered before and after azoxymethane, whereas its *N*-acetylcysteine conjugate inhibited colon tumour development when given after but not before azoxymethane. In another study, phenethyl-ITC was ineffective in reducing the number of azoxy-methane-induced aberrant crypt foci in rat colon. Phenethyl-ITC was highly effective in blocking 4-(methylnitro-samino)-1-(3-pyridyl)-1-butanone (NNK)- induced lung tumours in rats. Dietary phenethyl-ITC did not affect the incidence or multiplicity of mammary tumours induced in rats by 7,12-dimethylbenz[*a*]anthracene (DMBA) and did not reduce the number of papillomas induced in rat urinary bladder by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN).

Phenethyl-ITC potently inhibited lung tumours in mice when administered orally before treatment with NNK. Thiol and *N*-acetylcysteine conjugates of phenethyl-ITC were also effective. Phenethyl-ITC was ineffective when given after NNK. It did not prevent benzo[*a*]pyrene-induced tumours in mice, but benzyl-ITC and phenethyl-ITC administered in combination reduced the number of lung tumours induced by a combination of NNK and benzo[*a*]pyrene. Phenethyl-ITC did not inhibit lung tumours induced in mice by environmental tobacco smoke. It inhibited the development of forestomach tumours in mice given benzo[*a*]pyrene. When administered in the diet before and after *N*-nitrosodiethylamine, phenethyl-ITC inhibited the formation of foci and adenomas in mouse liver.

Phenethyl-ITC was a highly effective inhibitor of lung tumours induced in hamsters by *N*-nitrosobis(2-oxopropyl)amine (BOP). When administered orally before treatment of hamsters with BOP, phenethyl-ITC reduced pancreatic tumour development.

Benzyl-ITC administered in the diet before, during and after NMBA treatment moderately inhibited oesophageal tumour development in rats. Dietary benzyl-ITC inhibited induction of tumours in the small intestine and colon of rats by methylazoxymethanol and inhibited DMBA-induced mammary tumours in rats. Benzyl-ITC administered by gavage inhibited tumours of the forestomach in mice treated with benzo[*a*]pyrene in one study, but not in another. It did not inhibit lung tumours induced in mice by NNK but was effective against tumours induced by benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons.

3-Phenylpropyl-ITC potently inhibited induction of oesophageal tumours in rats when administered in the diet before, during and after NMBA treatment. When administered orally to mice before treatment with NNK, 3-phenylpropyl-ITC potently inhibited lung tumour formation. It was a highly effective inhibitor of BOP-induced lung tumours in hamsters but was ineffective in inhibiting BOP-induced tumours in hamster pancreas when administered before the carcinogen.

Oral administration of sulforaphane inhibited mammary tumorigenesis induced in rats by DMBA. Dietary sulforaphane inhibited benzo[*a*]pyrene-induced forestomach tumours in *nrf2* competent mice but not in *nrf2* null mice.

In studies with synthetic isothiocyanates, 6-phenylhexyl-ITC was highly effective in blocking NNK-induced lung tumours in rats. When given in the diet, it also reduced the number of papillomas induced in rat urinary bladder by BBN. 4-Phenylbutyl-ITC administered in the diet of rats before, during and after NMBA moderately inhibited oesopha-geal tumour development. 6-Phenylhexyl-, 4-phenylbutyl- and 5-phenylpentyl-ITC administered orally to mice before treatment with NNK potently inhibited lung tumours. The thiol and *N*-acetylcysteine conjugates of 6-phenylhexyl-ITC were also effective. 4-Phenylbutyl-ITC was a highly effective inhibitor of BOP-induced lung tumours in hamsters but was only moderately effective in inhibiting BOP-induced tumours in hamster pancreas when administered before the carcinogen. Dietary α-naphthyl-ITC inhibited both hepatocellular carcinoma development and nodular hyperplasia in the livers of rats exposed to *meta*-toluylenediamine, *N*-ethionine or *N*-2-fluorenylacetamide. In structure–function studies, the synthetic isothiocyanates 1-hexyl-ITC, 2-hexyl-ITC, 1-dodecyl-ITC, 1,2-diphenethyl-ITC and 2,2-diphenethyl-ITC inhibited NNK-induced lung tumours in mice, but 4-oxo-4-(3-pyridyl)butyl-ITC and 4-(3-pyridyl)butyl-ITC were ineffective.

Indoles

Indole-3-carbinol reduced the tumour response in a range of species and target organs and acted against most carcinogens examined when applied before and during the period of carcinogen administration as a blocking agent. For instance, concomitant treatment provided dose-responsive inhibition of hepatocellular carcinoma in rats or rainbow trout receiving aflatoxin B_1. At sufficient doses, indole-3-carbinol either suppressed or blocked mammary tumorigenesis induced in rats, usually with DMBA as the initiating agent. The compound effectively inhibited lung tumours in mice initiated with tobacco-specific nitrosamines as well as DMBA-induced skin carcinogenesis in mice. Prolonged treatment also reduced the number of 'spontaneous' mammary tumours in BALB/cfC3H mice harbouring the murine mammary tumour virus, cervical cancer in mice transgenic for human papilloma virus-16 and uterine adenocarcinoma in female Donyu rats.

3.3′-Diindolylmethane blocked tumour initiation in several models, including DMBA-initiated rat mammary tumours and benzo[*a*]pyrene-initiated forestomach tumours in mice. It also suppressed mammary tumour progression in rats previously initiated with DMBA.

Intermediate effect biomarkers

Whole cruciferous vegetables, extracts and isothiocyanates have been shown to modulate intermediate biomarkers of effect in a variety of experimental animal models. The effect on expression of phase I and II enzymes and on the formation of DNA adducts is generally consistent with predictions from in-vitro studies and with inhibition of tumours in animal models. The rather limited data on the antioxidant properties of cruciferous vegetables are, however, difficult to interpret. Extracts of cruciferous vegetables have been shown to have antioxidant effects in vivo, but other vegetables may also have these effects. Some glucosinolate breakdown products can selectively modulate cell proliferation and induce apoptosis in initiated cells in vivo, and these phenomena may provide a mechanism for tumour suppression.

The results of studies on the inhibition of carcinogen–DNA adduct formation in vivo by indole-3-carbinol have been remarkably consistent, inhibition being seen with a variety of carcinogens in several animal species (rat, mouse, rainbow trout), independently of the method used to quantify DNA adducts. In addition, indole-3-carbinol inhibited DNA adduct formation in both target and non-target organs and in peripheral white blood cells.

In almost all studies in rats in which enzyme markers of chemoprevention of carcinogenesis by indole-3-carbinol were evaluated, the markers predicted the outcome (inhibition of tumour formation). In several cases, induction of enzymes (phase I or phase II) by indole-3-carbinol could also be related to inhibition of carcinogen–DNA adduct formation, which is a more generally accepted intermediate marker of chemopreventive effects. Evaluation of enzyme markers appeared to be less useful in the rainbow trout model of aflatoxin B_1-induced liver tumours.

While the limitations of the trout model for aflatoxin B_1-induced liver carcinogenesis are well known, it has proven to be useful in studying inhibition of carcinogenesis by indole-3-carbinol, not only because of the extreme sensitivity of the trout liver to aflatoxin B_1, but also because it facilitates the study of dose–response relationships. The main chemopreventive property of indole-3-carbinol in this system is as an anti-initiator. It appears to act in this manner in rodents mainly by inducing pathways involved in the detoxification of carcinogens, thereby reducing the availability of substrate for activation pathways leading to DNA adduct formation.

There is little evidence from experiments in animals that the urinary 16α-hydroxyestrone:2-hydroxyestrone ratio could be used as a biomarker of an anti-estrogenic or anticarcinogenic effect of indole-3-carbinol.

In-vitro studies

Cruciferous vegetable juices prepared from homogenates induced NAD(P)H:-quinone oxidoreductase, a marker of phase II enzyme induction, due in part to the isothiocyanates and indoles formed from glucosinolates during homogenization. Isothiocyanates induce this quinone reductase by transcriptionally activating gene expression through the anti-oxidant response element, mediated by the transcription factor *Nrf2*, which is negatively regulated by Keap-1. Isothiocyanates are selective inhibitors of cytochrome P450 enzymes involved in carcinogen metabolic activation. The combination of inhibition of cytochrome P450s, induction of phase II enzymes such as GSTs and induction of NAD(P)H:-quinone oxidoreductase plays an important role in the chemopreventive effects of isothiocyanates in animals.

Isothiocyanates bind non-enzymatically and reversibly to protein and non-protein thiols; the reaction with GSH is also catalysed by GSTs. Isothiocyanate–GSH conjugates are expelled from cells, thereby depleting GSH, inducing oxidative stress and favouring the formation of isothiocyanate–protein adducts (thiocarbamoylated proteins). These adducts are implicated in the induction of enzyme expression associated with the activation of apoptosis. Apoptosis is associated with activation of caspases, MAP kinases—especially JNK-1—and mitochondrial dysfunction. Changes in function and enhanced proteasomal destruction of thiocarbamoylated proteins might mediate these effects.

Indole-3-carbinol and its derived compounds activate a range of drug-metabolizing enzymes, in particular CYP1A1. These compounds alter the metabolism of estrogen, increasing the 2-OH product and decreasing the16α-OH product, thus decreasing proliferation of estrogen-responsive cells. They inhibit growth and induce apoptosis in a range of cell types, both estrogen receptor-positive and -negative. These effects are often more marked in tumour cells than in their non-tumorigenic counterparts. The expression of many proteins relevant to growth inhibition and apoptosis can be modified by indole-3-carbinol and 3,3′-diindolylmethane. Derivatives of indol-3-carbinol are generally more potent than the parent compound. In vitro, indolo[3,2-*b*]carbazole appears to have the least favourable profile for a chemopreventive agent.

Other beneficial effects

Two large cohort studies showed a statistically significant inverse association between consumption of cruciferous vegetables and coronary heart disease and ischaemic stroke. One of the studies also showed an inverse association between broccoli consumption and cataract.

Isothiocyanates have been found to be effective bacteriostatic and bactericidal agents, and this characteristic has been used in food preservation. A recent study showed evidence of an effect of sulforaphane against *Helicobacter pylori* in a human cell line.

Carcinogenicity

Humans

Studies in humans do not provide evidence that cruciferous vegetables are carcinogenic.

Experimental animals

There is no evidence that cruciferous vegetables *per se* are carcinogenic, although a few studies provide some evidence that dietary cabbage might enhance tumour responses in the colon, pancreas, skin and spermatic cord of animals treated with carcinogens.

A number of studies in experimental animals showed that isothiocyanates can not only inhibit but also enhance chemically induced tumorigenesis in rats. Phenethyl-ITC and benzyl-ITC increased chemically induced preneoplastic lesions and tumours in rat bladder, and phenethyl-ITC increased the frequency of altered foci

in the liver. When given to hamsters in the diet after initiation, it increased the frequency of dysplastic lesions in the pancreas. The mechanisms of the enhancing effects of these two compounds have not been determined. The synthetic 6-phenylhexyl-ITC enhanced carcinogenesis in rat oesophagus and colon. The enhancement in the oesophagus appeared to be due to toxic effects leading to increased cell proliferation, as the compound increased tumour size and the activities of both COX-2 and LOX enzymes.

Isothiocyanates can also elicit carcinogenic effects in untreated animals. In a number of studies, phenethyl-ITC induced preneoplastic lesions and tumours, and allyl-ITC induced preneoplastic lesions and papillomas in rat bladder. Treatment with benzyl-ITC led to the development of preneoplastic bladder lesions. The mechanisms of these adverse effects have not been determined.

In a number of studies, prolonged treatment of carcinogen-treated animals with indole-3-carbinol enhanced, rather than suppressed, the tumour response in some organs, especially liver. Dietary indole-3-carbinol promoted hepatocellular carcinoma when given to trout previously initiated with aflatoxin B_1. The potency of indole-3-carbinol for this adverse effect exceeded its potency as a blocking agent, was proportional to the duration and dose, with no evident threshold, and was not reversed after treatment was stopped. Prolonged dietary administration of indole-3-carbinol to rats post-initiation promoted hepatic GST-P$^+$ biomarkers and enhanced thyroid tumour development. In a separate study in rats, administration of indole-3-carbinol post-initiation resulted in modest suppression of mammary tumour and aberrant crypt foci formation in the colon but strongly promoted hepatic GST-P$^+$ foci in the same animals. Studies in trout and rodents and in cultured mammalian cells in vitro showed that indole-3-carbinol or derived metabolites can have estrogenic as well as anti-estrogenic behaviour, depending on the organ, cell type, biomarker or pathway being examined. This may be one basis for the bi-directional behaviour of indole-3-carbinol in tumorigenesis.

Toxic effects

Humans

There is little evidence of acute effects in humans after ingestion of cruciferous vegetables. Reversible drug interactions have been described that are due either to the high vitamin K content of several *Brassica* vegetables or to modulation of the activities of enzymes responsible for the metabolism of xenobiotics. The possible contribution of goitrogenic compounds present in seeds, roots and leaves of cruciferous vegetables to endemic goitre in many parts of the world remains controversial. There is some evidence that intake of certain glucosinolates at a level exceeding 50 mg per day might have goitrogenic effects, but the complex interactions between long-term intake of possible goitrogens from cruciferous vegetables and dietary intake of iodine and possibly other nutrients are not well understood.

Experimental animals and in vitro

Animal species differ significantly in their sensitivity to the various toxic compounds formed from glucosinolates. Acute and subacute toxic effects are observed mainly in the thyroid, liver and kidney. Rats and pigs are more sensitive than other species to goitrogenic compounds, and effects on the thyroid have been described at doses of 3–5 µg per kg bw per day of goitrin (5-vinyl-2-thiooxazolidone). The mechanism of this action appears to be unrelated to iodine uptake into the thyroid.

Several isothiocyanates have been shown to be cytotoxic, genotoxic and mutagenic in vitro in both prokaryotes and eukaryotes. The doses required to induce DNA damage in rats and mice in vivo were, however, several orders of magnitude higher than the amounts found in human diets.

The available information on the mutagenicity of indoles is limited to nitrosated indoles. As vegetables are rich in nitrates, it is highly likely that nitrosated indoles are formed in the stomach.