Glucosinolates in Brassica: occurrence and significance as cancer-modulating agents

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Les glycosinolates

RÉSUMÉ

Les légumes brassicas comprennent toutes sortes de choux, choux de Bruxelles, broccolis, choux-fleurs, choux-raves, navets et rutabagas. Ils appartiennent à famille des crucifères et sont connus en botannique sous le nom de *Brassica*. Les brassicas sont fréquemment consommés par les hommes des cultures occidentales et orientales aussi bien que par les animaux. Aux Pays-Bas, on ne connaît pas précisément les quantités consummées par les hommes, mais plus de 36 g brassicas y sont disponibles par personne et par jour. La saveur typique des brassicas est en grande partie due à des molécules volatiles dérivées du glycosinolate. Il semble que la présence de glycosinolates dans les plantes pour la consommation humaine est limitée aux crucifères. La composition chimique et la présence de glycosinolates et de leurs produits de dégradation ont été étudiées. Il est possible que les niveaux de glycosinolates totaux dans les plantes dépendent de la variété, des conditions de culture, du climat et des pratiques agronomiques, alors que les niveaux dans une plante particulière varient selon les parties de la plante. Dans les plantes, les glycosinolates et leurs produits d'hydrolyse agissent comme des allélochimiques, qui sont des composés qui affectent les comportement, la santé et la croissance d'autres espèces.

Du point de vue de la toxicologie, tous les produits volatiles d'hydrolyse des glucosinolates sont intéressants. Parmi les problèmes liés à la consommation de ces composés, ceux affectant la thyroïde ont été étudiés de façon plus approfondie. Dans la plupart des études sur les animaux réalisées dans le passé, la goitrogénicité induite par les produits dérivés des glycosinolates s'est révélée être limitée à des situations de déficience en iode. Dans une étude sur la fonction thyroïdienne aucun effet des glycosinolates n'a pu être retrouvé (McMillan *et al*. 1986).

On a suggéré que les produits dérivés des glycosinolates deviennent mutagènes et cancérigènes après nitrosation. Dans une étude approfondie, on a recherché dans plusieurs brassicas hollandais leur potentiel à former directement des composés mutagènes de nitrosyl en réponse à un traitement de nitrite. Ce potentiel a été comparé avec les niveaux des glycosinolates. On a étudié la formation de produits nitrosés d'indole-3-acétonitrile, indole-3-carbinol et indole, en portant une attention particulière à la stabilité de ces produits. Ces études ont révélé que les composés indoliques présents dans les brassicas peuvent être nitrosés et devenir mutagènes à la nitrosation. Cependant les produits nitrosés ne sont stables qu'en présence de grandes quantités de nitrite libre. La nitrosation des glucosinolates et de leurs produits dérivés ne constitue pas un danger significatif pour la santé.

En revanche, le données épidémiologiques indiquent qu'une consommation de crucifères est associée à une réduction de l'incidence du cancer dans les populations humaines (Graham *et al.* 1978; Graham & Mettlin, 1979; Graham, 1983; Hirayama, 1986). Plusieurs principes actifs ont été identifiés comme produits dérivés de glycosinolates, et il a été montré que l'explication probable des effets protecteurs observés est l'induction d'enzymes du système de biotransformation. L'effet protecteur semble être dû à une modification dans l'équilibre entre les systèmes enzymatiques activants (associés aux cytochromes P450) et les systèmes enzymatiques désactivants (conjugants) favorisant la désactivation de mutagènes et de cancérigènes connus.


OCCURRENCE

Brassica vegetables comprise all kinds of cabbages, Brussels sprouts, broccoli, cauliflower, kohlrabi, turnip and swede. They belong to the family of Cruciferae, and are botanically known as Brassica species. Brassicas are frequently consumed by humans from both Western and Eastern cultures (Fenwick & Heaney, 1983; Hill *et al.* 1987), as well as by animals. In the Netherlands, the amounts consumed by humans are not known exactly, but more than 36 g brassica per person per d are available (Godeschalk, 1987).

The typical flavour of brassicas is largely due to glucosinolate-derived volatiles. The occurrence of glucosinolates in plants for human consumption appears to be limited to cruciferous plants. The chemistry and occurrence of glucosinolates and their breakdown products has been reviewed extensively by van Etten & Wolff (1973) and by Fenwick *et al.* (1983). Glucosinolates contain a common structure (Fig. 1), with different substituents (alkyl, alkenyl, alkylthioalkyl, aryl, β-hydroxyalkyl or indolylmethyl groups) at the side chain. Currently more than 100 different glucosinolates have been characterized. The levels of total glucosinolates in plants may depend on variety, cultivation conditions, climate and agronomic practice, while the levels in a particular plant vary between the parts of the plant. Table 1 gives data for the types and levels of glucosinolates occurring in Dutch vegetables (Tiedink *et al.* 1988).

In plants glucosinolates and their hydrolysis products act as allelochemicals, which are compounds affecting behaviour, health and growth of other species. For instance, the white butterfly, *Pieris rapae*, is attracted to brassicas, and probably needs glucosinolates for essential functions, while in general, glucosinolates and their hydrolysis products also have insecticidal and fungicidal properties. In higher plants the hydrolysis product of indolylglucosinolates, indole-3-acetonitrile (I3A) and indole-3-acetic acid formed from I3A (Gmelin & Virtanen, 1961), also act as hormones (auxins).

Glucosinolates can be hydrolysed by myrosinase (thioglucoside glucohydrolase; *EC* 3.2.3.1), an enzyme also present in Cruciferae, which becomes active when cells are damaged, e.g. by cutting or chewing. Myrosinase hydrolyses glucosinolates by splitting off the glucose, the unstable aglucone then eliminates sulphate by a Lossen rearrangement (Fig. 2). The structure of the resulting products depends on a variety of factors, but in general isothiocyanates, nitriles and thiocyanates are formed. Less-volatile compounds such as epithionitriles and oxazoldine-2-thiones are formed from glucosinolates with an hydroxyl group at the 2-position of the side chain. Myrosinase can be inactivated by heating, although equivocal data exist about the levels of inactivation. Bradfield & Bjeldanes (1987) only found a 20% reduction in myrosinase activity after
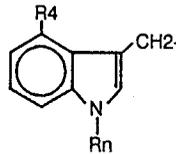
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        S—ß-glucose
   R—C
        N—sulphate
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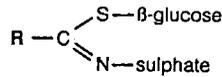
R = Alkyl:

C=C-C-	Sinigrin
C=C-COH-C-	Progoitrin
C=C-C-C-	Gluconapin
C-SO-C-C-C-	Glucoiberin
C-SO-C-C-C-C-	Glucoraphanin
C-SO-C-C-C-C-C-	Glucoalyssin
C-S-C=C-C-C-	4-Methylthiobutenyl-glucosinolate

R = Aryl:
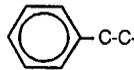
⬡-C-C-   Glucosturtiin

R = Indolyl:

R4	Rn	
H	H	Glucobrassicin
OH	H	4-Hydroxy-glucobrassicin
OCH3	H	4-Methoxy-glucobrassicin
H	OCH3	Neoglucobrassicin

Fig. 1. Chemical structure of the most commonly occurring glucosinolates in cruciferous vegetables.

Table 1. *Types and levels of glucosinolates occurring in Dutch vegetables*
(From Tiedink *et al.* 1988)

	Glucosinolates (μmol/g dry wt vegetable)															
	Aryl- and alkyl-glucosinolates										Indolyl-glucosinolates					Total
Vegetables	1	2	3	4	5	6	7	8	9	Total	10	11	12	13	Total	(1–13)
Cauliflower	0·2	–	0·2	–	0·3	–	–	–	–	0·7	0·1	0·7	0·2	0·1	1·1	1·8
Brussels sprouts	3·1	7·6	8·2	2·1	0·3	7·0	0·3	–	–	28·3	1·2	4·5	0·9	–	6·6	34·9
Savoy cabbage	4·3	0·3	4·3	0·4	0·3	0·4	–	–	–	10·0	0·4	2·4	1·8	0·1	4·7	14·7
Broccoli	0·5	6·2	–	5·9	0·3	0·6	–	–	–	13·5	–	2·1	0·2	0·9	3·2	16·7
Red cabbage	1·6	1·2	2·7	0·3	0·1	0·4	–	–	–	6·2	0·1	3·8	0·3	–	4·2	10·4
Green cabbage	7·3	0·2	10·2	–	0·2	–	–	–	–	17·9	–	6·8	1·3	–	8·1	26·0
Oxheart cabbage	0·7	0·3	0·1	0·9	0·3	–	–	–	–	2·3	0·1	1·1	0·1	–	1·3	3·6
White cabbage	6·8	0·2	4·2	0·1	0·2	–	–	–	–	11·5	–	3·4	0·4	0·1	3·9	15·4
Kohlrabi	0·2	–	–	0·2	0·1	–	–	–	–	0·5	0·1	1·3	0·1	0·5	2·0	2·5
Chinese cabbage	–	–	–	–	–	–	0·5	–	–	0·5	–	1·3	1·5	0·1	2·9	3·4
Swede	–	2·3	–	–	0·7	–	2·9	–	0·5	6·4	0·2	0·9	0·3	0·9	2·3	8·7
Radish	–	0·2	–	–	0·2	–	–	4·9	0·7	6·0	0·2	0·3	0·5	–	1·0	7·0
Horseradish	–	–	–	–	0·2	–	–	9·6	0·4	10·2	0·3	0·3	0·1	–	0·7	10·9

1, Glucoiberin; 2, progoitrin; 3, sinigrin; 4, glucoalyssin; 5, glucoraphanin; 6, gluconapin; 7, glucosturtiin; 8, 4-methylthiobutenylglucosinolate; 9, the rest of the aryl- and alkyl-glucosinolates; 10, 4-hydroxyglucobrassicin; 11, glucobrassicin; 12, 4-methoxyglucobrassicin; 13, neoglucobrassicin.

cooking cauliflower (*Brassica oleracea*) for 10 min, while McMillan *et al.* (1986) detected only about 0–2·5% of its original activity in Brussels sprouts after cooking for 9 min. Slominsky & Campbell (1987) found complete inactivation of myrosinase when rapeseed (*Brassica napus*) meal was heated to 95° for 10 min, although substantial decomposition of glucosinolates was observed during heating. Glucosinolates are lost during cooking of cruciferous vegetables due to leaching into the cooking liquor (Srisangham *et al.* 1980; Fenwick *et al.* 1983). The effects of processing on glucosinolate levels in vegetables have been reviewed by de Vos & Blijleven (1988). On hydrolysis of indolylglucosinolates (Fig. 3) unstable isothiocyanates are formed which degrade to indole-3-carbinol (I3C), while under more acidic conditions I3A and elemental S are formed. I3C may condense to diindolylmethane (DIM) or react with ascorbic acid to form ascorbigen (ASC).

GLUCOSINOLATE-MEDIATED TOXIC EFFECTS

Goitrogenic effects

From a toxicological point of view all the volatile hydrolysis products of glucosinolates are of interest. Among the problems associated with the consumption of these compounds, those affecting the thyroid have been studied most extensively (van Etten, 1969). Thiocyanate ions and oxazolidine-2-thiones are goitrogenic in animals, although the mechanisms involved seemed to be different. Thiocyanate ions are considered to behave as iodine competitors and, therefore, will cause goitrogenicity only in cases of iodine deficiency, while oxazolidine-2-thiones interfere with thyroxine synthesis and, therefore, will be goitrogenic irrespective of the iodine status. Additionally, some isothiocyanates and nitriles may also lead to goitrogenic symptoms resulting from the thiocyanate-mediated detoxification process. In most animal studies performed in the
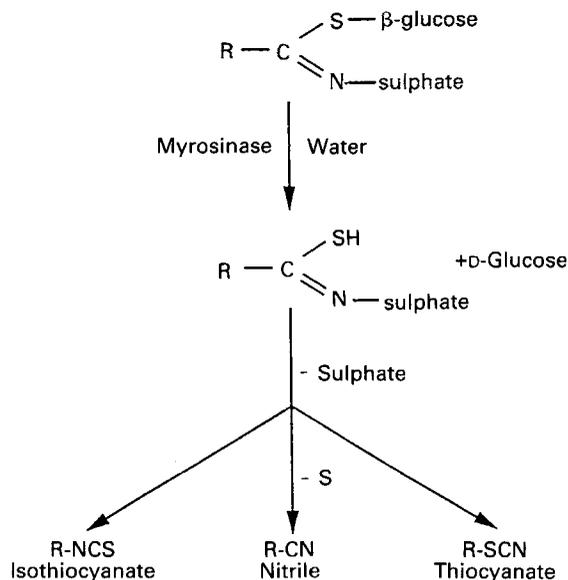

Fig. 2. Common structure of glucosinolates and their breakdown products formed on myrosinase (*EC* 3.2.3.1) hydrolysis.
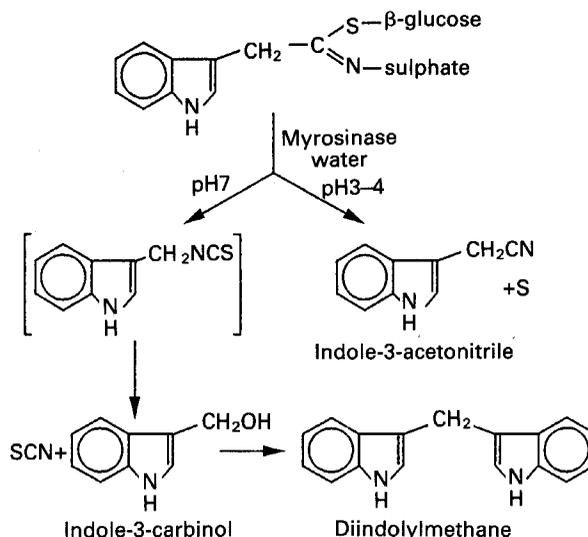
Fig. 3. The breakdown products of indolylglucosinolates on myrosinase (*EC* 3.2.3.1) hydrolysis.

past, goitrogenicity induced by glucosinolate breakdown products appeared to be limited to situations of iodine deficiency. In a study of McMillan *et al.* (1986), no effects on human thyroid function could be found, as measured by the levels of thyroid-stimulating hormone, triiodothyronine and thyroxine after a daily consumption of 150 g Brussels sprouts over a period of 9 weeks.

In addition to the effects of glucosinolate breakdown products on the thyroid other effects have been observed. Hydroxynitriles and glucosinolates themselves were suggested to be responsible for enlargement of adrenal glands, kidneys and liver in rats after long-term feeding of rapeseed and for liver haemorrhage observed in poultry. Several isothiocyanates have been shown to be embryotoxic in rat, while in *in vitro* studies a number of them appeared to be cytotoxic and mutagenic. Moreover, allylisothiocyanate is able to induce tumours in rats (National Toxicological Programme, 1981).

Nitrosation of glucosinolates

In many studies the presence of large amounts of compounds susceptible to nitrosation in food products was investigated by nitrosation of extracts of these food products followed by total N-nitroso compound (NOC) determination or by measurement of alkylating and/or mutagenic activity. In most of these studies the experimental conditions during nitrosation were modifications of the so-called nitrosation assay procedure which is the test protocol adopted by the World Health Organization to investigate the occurrence of compounds susceptible to nitrosation in drugs. After nitrite treatment sauerkraut, pickled vegetables, Chinese cabbage (*Brassica pekinensis*) and fava beans (*Vicia faba*) contained direct mutagenic activity (Piacek-Llanes & Tannenbaum, 1982; Wakabayashi *et al.* 1983, 1984, 1985; van der Hoeven *et al.* 1984; Jongen *et al.* 1987; Groenen & Bussink, 1988). Further work showed that I3A, 4-methoxyindole-3-acetonitrile and 4-methoxyindole-3-aldehyde could act as precursors of NOC in Chinese cabbage (Wakabayashi *et al.* 1985, 1986).

Since these indole compounds are hydrolysis products of glucosinolates Tiedink *et al.* (1988, 1989, 1990, 1991) paid special attention to the human relevance of the role of indole compounds and glucosinolates in the formation of NOC in vegetables. Vegetables commonly consumed in the Netherlands were screened for their potential to form directly mutagenic NOC on nitrite treatment. For brassicas this potential was compared with their glucosinolate levels. The formation of nitrosated products of I3A, I3C and indole were studied and special attention was paid to the stability of these products. After investigation of the hydrolysis of purified glucosinolates by myrosinase and acid, the contribution of intact and hydrolysed glucosinolates, as well as several known indole compounds, to the total mutagenicity of nitrite-treated brassica extracts was studied. These studies revealed that the indole compounds present in Brassica vegetables can be nitrosated and thereby become mutagenic. However, the nitrosated products are stable only in the presence of large amounts of free nitrite. If the food matrix is present during the nitrosation procedure glucosinolates and their breakdown products contribute only marginally to the presence of NOC in these vegetables. Consequently nitrosation of glucosinolates and their breakdown products does not constitute a significant health risk.

GLUCOSINOLATES AS CANCER-MODULATING AGENTS

The process of tumour formation

There is abundant epidemiological and experimental evidence that the process of carcinogenesis is a multi-stage process in which at least three distinct phases can be recognized: the initiation phase, the promotion phase and the progression phase (Ponten, 1987). Initiation is the genetic event which is considered to be the first step in the onset of carcinogenesis. Although a chemical that can bind covalently with DNA will do so largely at random, only specific lesions will result in genetic alterations that are relevant for the cancer process, such as activation of oncogenes. The initiation phase comprises two distinct steps: the induction of the molecular lesions and the fixation of these lesions by DNA replication. Promotion is the process whereby an initiated tissue or organ develops focal proliferations of phenotypically altered cells. In its first phase promotion is reversible and promoters appear to act only above a certain threshold level. Promotion can be brought about by aspecific stimuli such as wounding, bacterial infection, cell killing, partial hepatectomy etc. In addition to this aspecific type of effect, promotion can be brought about by specific mitogenic stimuli. For example, growth factors and hormones can act as promoters and other types of promoters can interfere with growth factor pathways, for example, by influencing receptor affinity and binding sites. *In vitro* studies have shown that many of these proliferative stimuli are triggered by promoter–membrane interactions. Structural and functional changes in cellular membranes have been reported, such as activation or inhibition of plasma-membrane-bound enzymes, modulation of ion transport and transport of small molecules through plasma membranes (Slaga, 1984; Yamasaki, 1984). One major aim of studies on tumour promotion is to determine which components of a cell society are responsible for maintaining dormancy of initiated cells and, as a corollary, how tumour promoters interfere with such societal influence to permit clonal expansion. In the progression phase, one or more of the focal proliferations undergo a cellular evolution to malignant neoplasm. During this evolution these cells acquire several characteristics which can be divided into the following categories:

infinite growth: there is an indefinite expansion of the net cell number in a tumour, resulting in a progressive increase in tumour burden;

genetic instability: within a tumour new abnormal genotypes are created and fixed, implying a considerable risk of losing important proliferation and differentiation controls;

irregular migration: cells acquire invasive properties, which means that they have the potential to migrate, settle and grow at abnormal locations in the organism.

In experimental animal studies progression is accomplished by addition of initiating agents which induce additional genetic changes.

Role of biotransformation

Living organisms are continually exposed to a variety of naturally-occurring chemicals. If these compounds are lipophilic they are generally made more hydrophilic by metabolic transformations, mainly oxidations or hydroxylations, and subsequent conjugations to facilitate excretion. This process of metabolic transformation can be divided into two phases. Phase 1 reactions introduce new functional groups into the lipophilic compounds, converting them into highly reactive products which can bind covalently to nucleophilic molecules. Phase 2 metabolism comprises synthetic reactions with small endogenous molecules that are coupled to functional groups originally present or introduced by phase I metabolism. The ability of carcinogens to exert their effects depends largely on the balance between activating and de-activating enzymes. Any change in this balance will result in a change in the biological effect. Many compounds occurring naturally in the human diet modulate the biotransformation of several carcinogens, resulting in reduced tumour incidence (Wright, 1980). This implies compounds which inhibit the formation of electrophilic intermediates by acting as inducers of alternative pathways, as well as compounds which change the balance between activating and de-activating enzyme systems (van Bladeren, 1993). The liver is the organ predominantly involved in the biotransformation of many xenobiotics. For practical purposes, in the majority of *in vitro* studies, liver homogenates or subcellular fractions have been used to mimic *in vivo* metabolism. The use of this kind of preparation has several disadvantages. Due to homogenization and fractionation of cells the balance between activating and de-activating enzyme systems is disturbed. In particular, conjugation reactions, which are located in different cellular compartments, are much less operative (Moldeus, 1981). This kind of disadvantage may be overcome by the use of intact cells as a metabolizing system. Isolated hepatocytes resemble the *in vivo* situation in their structural and biochemical properties much more closely than subcellular fractions (Wortelboer *et al.* 1990). When freshly-isolated hepatocytes are cultured they show a rapid decline in their capacity for biotransformation. The various isozymes of the cytochrome P450 family, especially, show a rapid loss of activity. To overcome this problem several methods have been applied, such as addition of hormones, addition of enzyme inducers and co-culture with epithelial cells or on extracellular membranes. Although some of these methods were partially successful in retaining the total cytochrome P450 activity, selective changes were observed in the pattern of different cytochrome P450 isozymes (Althaus *et al.* 1979). In contrast to parenchymal cells isolated from mammalian liver, chick-embryo hepatocytes maintain their initial levels of cytochrome P450 enzyme activities *in vitro* for at least 3 d (Topp &

van Bladeren, 1986). Moreover, induction with known enzyme inducers such as phenobarbital, 3-methylcholanthrene or Aroclor 1254 resulted in more than a two-fold increase in cytochrome P450 content (Jongen *et al.* 1987). Also when this system was used to study modulating effects of dietary components such as several indole compounds and the synthetic flavonol β-naphthoflavone, treatment of cultured chick-embryo hepatocytes with these compounds resulted in increases in activities of both phase I and phase II enzyme systems.

Glucosinolates and cancer prevention

Epidemiological data indicate that consumption of cruciferous vegetables is associated with a decreased incidence of cancer in human populations (Graham *et al.* 1978; Graham & Mettlin, 1979; Graham, 1983; Hirayama, 1986). The anti-carcinogenic properties of cruciferous vegetables and isolated compounds have been studied in several investigations. When animals were first fed on diets high in cruciferous vegetables and then exposed to various indirect-acting carcinogens, tumour yields were lower and survival rates were higher than similarly-treated animals on semi-purified diets (Stoewsand *et al.* 1978; Boyd *et al.* 1982; Wattenberg, 1983). A number of these cruciferous plants, including Brussels sprouts, cabbage, cauliflower and broccoli have been found to induce aryl hydrocarbon hydroxylase (*EC* 1.14.14.1) activity (Wattenberg *et al.* 1976) and/or the activity of cytosolic glutathione-S-transferase (*EC* 2.5.1.18) (Sparnins *et al.* 1982). Subsequently, isolation and identification of the principal compounds involved showed that they were indoles (Loub *et al.* 1975). When the identified compounds were tested, I3C, I3A, DIM and ASC were shown to inhibit neoplastic effects of carcinogens (for review, see McDanell *et al.* 1988). However, some specific differences were observed; I3C, DIM and I3A all appeared to inhibit the induction of forestomach neoplasia by benzo(a)pyrene (B(a)P). I3C and DIM also inhibited 7,12-dimethylbenzanthracene-induced mammary tumours, although I3A did not (Wattenberg & Loub, 1978). Initially these effects were thought to be the result of induction of enzymes of the mixed function oxidase (MFO) system (Loub *et al.* 1975), which can be induced by intact glucobrassicin as well (McDanell *et al.* 1989). Later, I3C was also found to be an inducer of glutathione-S-transferase, glucuronyl transferase and epoxide hydroxylase (*EC* 3.3.2.3), enzymes involved in the detoxification of xenobiotics (Sparnins *et al.* 1982; Cha *et al.* 1985). The mode of action of this type of anti-carcinogenic compound thus seems to involve a qualitative and quantitative change in the metabolism of the carcinogens (Wattenberg, 1993). In contrast to all these findings, Pence *et al.* (1986) reported that I3C enhanced dimethylhydrazine-induced tumour formation. Also, indole compounds can enhance the formation of tumours in trout (*Salmo fario*) when administered after a carcinogen (Bailey *et al.* 1987). Apparently the type of modulation is largely dependent on the choice of the model carcinogen.

So far *in vitro* data on the protective effects of pretreatment with indoles against the DNA-damaging capacity of carcinogens are scarce. Probably this is due mainly to the fact that in most *in vitro* systems it is not possible to induce biotransformation enzymes. The use of *in vitro* cultures of primary chick-embryo hepatocytes may offer a useful model to study modulating effects of indoles. These hepatocytes maintain their biotransformation capacity *in vitro* for up to 72 h without loss of activity. Moreover, induction with known enzyme inducers leads to enzyme induction comparable with that occurring in *in vivo* situations (Althaus *et al.* 1979; Giger & Meyer, 1981; Topp & van Bladeren, 1986).

A co-culture system to study mechanisms of glucosinolate-mediated modulation of biotransformation systems

The system consists of two cell types, primary chick-embryo hepatocytes and V79 Chinese hamster cells. Primary chick-embryo hepatocytes are isolated from 15-d-old chick embryos and cultured for 24 h. After treatment for a specific time with the modulating compound, the compound is removed and V79 cells are added. After 2 h the mutagen is added and the cells are exposed. If simultaneous treatment is wanted the modulating compound is added together with the mutagen. After exposure the cells are dissociated and the V79 cells are used for determination of changes in the genetic end-points, for example, by plating on microscopic slides for sister chromatid exchange determination or in Petri dishes for measurement of hypoxanthine-guanine phosphoribosyltransferase (*EC* 2.4.2.8) mutants (Jongen *et al.* 1989; Jongen, 1993). Using this approach, detailed studies were carried out to investigate the mechanisms underlying observed protective effects of various modulating compounds on B(a)P-mediated mutagenicity (Jongen, 1988). Treatment with I3C, I3A and β-naphthoflavone resulted in increases in cytochrome P450-associated enzyme activities. When metabolite formation of B(a)P was studied, large increases were observed in the amounts of specific metabolites. However, no shift was found in the ratios between the various metabolites when compared with metabolite formation following treatment with the carcinogen 3-methylcholanthrene. Formation of the mutagenic and carcinogenic metabolite, B(a)P-7, 8-dihydrodiol, did not differ between the three compounds. This indicates that induction of cytochrome P450-associated enzyme activity as such is not an appropriate indicator for modulating capacity. However, pretreatment with I3C and β-naphthoflavone followed by exposure to B(a)P resulted in decreased mutagenic effects (Table 2). Experiments with subcellular fractions showed that the mechanisms underlying these protective effects were different for the respective compounds. In the case of I3C the protective effects result from a changed balance of the enzyme systems involved in the I3C biotransformation process. There is an overall increase in the rate of metabolism, but the increases in conjugating enzyme activity compensate for the increases in cytochrome P450-associated enzyme activities. In the case of β-naphthoflavone the available data strongly suggest that the inhibitory effects cannot be ascribed to the inducing capabilities of the compound, but instead seem to be due to the formation of an

Table 2. *Inhibitory effect of pretreatment with various enzyme-inducing agents on genotoxicity of some carcinogens* (Data from Jongen, 1988)

(Experiments performed using co-culture system)

| | Exposure | | Reduction (%) in SCE per chromosome Pretreatment with: | | |
Compound	Dose (μg/ml)	Time (h)	I3C	I3A	β-NF
B(a)P	5·0	20	33	30	44
DMNA	1500	4	46	–	–
2-AA	10·0	20	–	–	–

I3C, indole-3-carbinol; I3A, indole-3-acetonitrile; β-NF, β-naphthoflavone; B(a)P, benzo(a)pyrene; DMNA, dimethylnitrosamine; 2-AA, 2-aminoanthracene; SCE, sister chromatid exchange.

intracellular pool of β-naphthoflavone which acts as a competitive inhibitor for B(a)P metabolism.

Using enzyme preparations from rat liver and from chick-embryo liver the metabolic fates of the indole compounds I3C and I3A were also studied. The metabolites formed were isolated and identified by GC-MS and NMR studies. It appeared that from both compounds the same metabolites were formed. A first step in this metabolism was the formation of indole-3-carboxaldehyde. The metabolic route resulting in the formation of this intermediate metabolite was different for both compounds. In the case of I3C the microsomal cytochrome P450 system (MFO in Fig. 4) and the cytosolic alcohol dehydrogenase system (ADH) were involved, whereas in the case of I3A only the cytochrome P450 enzyme system appeared to be operative. This difference explains why I3C is metabolized much faster than I3A. The subsequent step was identical for both compounds. Metabolism by the cytochrome P450 system resulted in the formation of the 5-hydroxy-indole-carboxaldehyde metabolite as well as in the formation of the carboxylic acid. The latter metabolite was also formed by the ADH system (Fig. 4). A comparative study between the two types of microsomal preparations showed that there were no differences in metabolic routing. If the same system is operative in the human situation these metabolites could possibly be used as biomarkers.

Problems associated with the study of cancer-modulating compounds

Selection of mutagen or carcinogen. In 1982 the National Research Council in the USA recommended increased consumption of cruciferous vegetables (National Toxicological
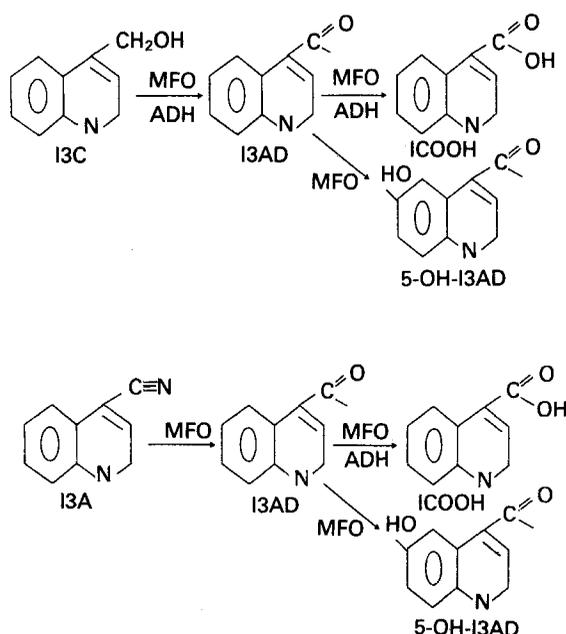

Fig. 4. Metabolites formed from indole-3-carbinol (I3C) and indole-3-acetonitrile (I3A) by microsomal preparations from rat. I3AD, Indole-3-carboxaldehyde; ICOOH, indole-3-carboxylic acid; 5-OH-I3AD, 5-hydroxy-indole-carboxaldehyde; MFO, mixed function oxidase system; ADH, alcohol dehydrogenase system.

Table 3. *Modulating effects of indole-3-carbinol pretreatment on sister chromatid exchange (SCE) induction by mutagens from different chemical classes* (Data from Jongen *et al.* 1989)

(Experiments were performed using a co-culture system of primary chick-embryo hepatocytes and V79 Chinese hamster cells)

| | Exposure | | SCE per chromosome | | |
Compound	Time (h)	Dose (μg/ml)	Without pretreatment	With pretreatment	Change (%)
B(a)P	24	7·5	1·46	1·07	−33
DMNA	4	1500	0·91	0·48	−46
2-AA	4	25·0	0·81	0·87	
DBE	4	5·3	1·11	1·45	+40
EMS	24	100	1·04	1·02	
Control	24	0	0·27	0·28	

B(a)P, benzo(a)pyrene; DMNA, dimethylnitrosamine; 2-AA, 2-aminoanthracene; DBE, dibromoethane; EMS, ethylmethanesulphonate.

Programme, 1982). At that stage the exact mechanism of the protective effect was not known. Later, several active principles were identified as breakdown products of glucosinolates and it was shown that the probable explanation for the observed protective effects is the induction of enzymes of the biotransformation system. Treatment with the isolated compounds or the vegetables followed by exposure to known carcinogens caused a shift in the balance between activating and conjugating enzyme systems favouring conjugation and resulting in a decrease of the biological activity of the carcinogen. This is an important observation since the majority of known carcinogens are not carcinogenic *per se* but have to be activated by enzymes of the biotransformation system to become biologically active. This also has important consequences for the conclusions which can be drawn from this type of experiment. Bioactivation of carcinogens from different chemical classes occurs through different biochemical pathways and in the case of halogenated compounds can be brought about also by conjugating enzymes (Jongen *et al.* 1982; van Bladeren *et al.* 1993). In the case of the brassica compounds exposure to carcinogens which are activated by conjugating systems will lead to increased effects instead of reduced effects. Apparently the outcome of this type of study depends largely on the choice of the carcinogen to which the animals are exposed. In Table 3 an example is given of the effects of pretreatment with I3C, one of the breakdown products of glucobrassicin, on the mutagenic effects of compounds from different chemical classes. Mutagenicity was determined by measurement of the number of sister chromatid exchanges. Using the co-culture system consisting of primary chick-embryo hepatocytes and V79 Chinese hamster cells it was shown that in the case of B(a)P and dimethylnitrosamine pretreatment with I3C resulted in decreased biological activity, whereas in the case of dibromoethane pretreatment resulted in enhanced mutagenic effects. From these observations it is clear that the choice (of combinations) of the appropriate model carcinogen(s) is a crucial step in the setup of experimental research.

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