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Glucosinolates in the human diet. Bioavailability and implications for health

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Abstract

The glucosinolates are a large group of sulphur-containing glucosides found in brassica vegetables. After physical damage to the plant tissue, glucosinolates are broken down, by the endogenous enzyme myrosinase, releasing glucose and a complex variety of biologically active products. The most important and extensively studied of these compounds are the isothiocyanates. Glucosinolates can be degraded or leached from vegetable tissue during food processing, but thermal inactivation of myrosinase preserves some intact glucosinolates in cooked vegetables. Once ingested, any remaining intact glucosinolates may be broken down by plant myrosinase in the small intestine, or by bacterial myrosinase in the colon. Isothiocyanates are absorbed from the small bowel and colon, and the metabolites are detectable in human urine 2–3 h after consumption of brassica vegetables. Isothiocyanates are potent inducers of Phase II enzymes *in vitro*, and they have been shown to increase the metabolism and detoxification of chemical carcinogens *in vitro* and in animal models. Some of these compounds also inhibit mitosis and stimulate apoptosis in human tumour cells, *in vitro* and *in vivo.* This second effect raises the possibility that in addition to blocking DNA damage, isothiocyanates may selectively inhibit the growth of tumour cells even after initiation by chemical carcinogens. Epidemiological evidence supports the possibility that glucosinolate breakdown products derived from brassica vegetables may protect against human cancers, especially those of the gastrointestinal tract and lung. To define and exploit these potentially anticarcinogenic effects it is important to understand and manipulate glucosinolate chemistry and metabolism across the whole food-chain, from production and processing to consumption.

Introduction

Plant foods provide micronutrients, dietary fibre, and an immense variety of biologically active secondary metabolites (Johnson et al., 1994). A large number of cohort and case-control studies have established that a high consumption of cabbages, broccoli, cauliflower, and Brussels sprouts leads to a decreased risk of carcinomas of the lung, stomach, colon and rectum (van Poppel et al., 1999). Plant families of the order Capparales, which includes all the Brassica vegetables, are characterised by the presence of the glucosinolates, of which more than 100 have now been identified (Fenwick et al., 1983). All the glucosinolates possess a common structure comprising a sulphonated moiety, a β-D-thioglucose group, and a variable side-chain derived from methionine, tryptophan, phenylalanine or various branched chain amino acids (Mithen et al., 2000). The glucosinolates are chemically stable and biologically inactive whilst they remain sequestered within sub-cellular compartments throughout the plant. However tissue damage caused by pests, harvesting, food processing or chewing initiates contact with the endogenous enzyme myrosinase, (thioglucoside glycohydrolase; EC 3.2.3.1). This leads to rapid hydrolysis of the glucosidic bond, releasing glucose and an unstable intermediate, which undergoes a spontaneous rearrangement to form a complex variety of breakdown products. The isothiocyanates, a group of hot and bitter compounds, commonly called *mustard oils* are probably the most important and thor-

oughly investigated of these products (Fenwick et al., 1982).

The bioavailability of glucosinolate breakdown products in the human diet

The levels of glucosinolates ingested depend on variety, agronomic factors, and both storage and processing of the vegetables prior to consumption (Mithen et al., 2000). Although mechanical damage leads to the rapid hydrolysis and degradation of glucosinolates, cutting and pre-harvest stress has also been shown, under certain circumstances, to increase the concentrations of indole glucosinolates in cabbage (Verkerk et al., 2001). The history of the plant tissue throughout the whole food chain from grower to consumer is therefore a vital influence on its ultimate biological role in human nutrition.

Whatever the final level of glucosinolates in the prepared vegetable, the absorption, metabolism and delivery of glucosinolate breakdown products to target tissues depends, to a large extent, upon the residual level of myrosinase activity (Dekker et al., 2000; Mithen et al., 2000). The activation of myrosinase is brought about by the physical disruption of the plant tissue during harvesting, processing, food preparation and consumption. However chopping induces glucosinolate hydrolysis only at the cut surfaces. Thus large intact leaves, or florets of broccoli or cauliflower, will undergo only minimal losses of glucosinolates up to the point of cooking. If such vegetables are eaten raw, both intact glucosinolates and active myrosinase are ingested simultaneously, which enables the breakdown of the glucosinolates to occur within the alimentary tract. For example, when rats were fed benzyl glucosinolate in the presence of active myrosinase derived from Brussels sprouts, a substantial proportion of the administered dose appeared as isothiocyanate excretion products in the urine (Rouzard et al., 2000). Some of the ingested glucosinolates were also broken down in the colon, but plant myrosinase appeared to be the dominant factor. Getahun and Chung (1999) observed a similar phenomenon in human subjects, using watercress as a source of PEITC. Volunteers consumed 350 g (475 μmol glucosinolates) of watercress in which the myrosinase had been completely inactivated by cooking, or 150 g (972 μmol glucosinolates) of raw watercress, which retained its myrosinase activity. In the case of cooked watercress, the rate of conversion of glucosinolates to isothiocyanates ranged from 1.2–7.3%, compared to 17.2–77.7% for the raw plant material.

Given the considerable loss of glucosinolates that results from cooking in water, it is clear that the method of preparation can make a very large difference, both to the intake of glucosinolates, and to the bioavailability of their breakdown products. Cooking reduces the concentration of glucosinolates in the plant tissue through thermal breakdown and leaching, but also inhibits the activity of myrosinase through denaturation of the enzyme. A mathematical model describing these effects has recently been developed by Dekker and co-workers, and this opens up the possibility of quantifying the losses of glucosinolates, and by extension other phytochemicals, during the storage, processing and culinary preparation of vegetables (Dekker et al., 2000).

The bacterial microflora of the human colon also express myrosinase activity. Significant quantities of isothiocyanate metabolites are excreted in the urine of healthy human volunteers after eating brassica vegetables, even when myrosinase has been completely inactivated by cooking (Getahun and Chung, 1999; Shapiro et al., 1998), however the excretion falls to negligible levels when the numbers of colonic bacteria are reduced by antibiotics (Shapiro et al., 1998). Rabot et al. (1995) isolated a strain of *Bacteroides thetaiotaomicron* (II8) from human faeces, which was capable of degrading glucosinolates. Inoculation of previously germ-free rats with this bacterium caused a considerable increase in the excretion of glucosinolate metabolites, and a correspondingly lower faecal excretion of intact sinigrin (Elfoul et al., 2001). This is clear confirmation that bacterial myrosinase activity can cause the breakdown of glucosinolates in the distal gut, leading to release of isothiocyanates into the faecal stream (Krul et al., 2002).

Presumably this mechanism accounts for the absorption and metabolism of isothiocyanates from thoroughly cooked brassica vegetables observed in human studies (Getahun and Chung, 1999). However, *in vitro* studies have recently shown that glucosinolate breakdown products themselves undergo complex patterns of further metabolism in complex bacterial systems, and further research will be necessary to determine whether this also occurs in humans *in vivo* (Combourieu et al., 2001).

Clearly the relative concentrations of glucosinolates and myrosinase present in brassica vegetables prepared for human consumption will vary in a complex way determined by aspects of the the food pro-

duction chain, all the way from the farm to the cooked food (Dekker et al., 2000). The main site of absorption within the upper gastrointestinal tract is not entirely clear, but this intraluminal digestion of glucosinolates, mediated not by endogenous enzymes but by plant myrosinase, appears to be the major route for the delivery of isothiocyanates, and possibly other breakdown products such as nitriles, to the circulation. In the absence of myrosinase activity, some intact glucosinolates are thought to be absorbed from the human alimentary tract, but the biological significance of this is unknown (Elfoul et al., 2001).

Biological effects of glucosinolate breakdown products

Phase I enzymes such as the cytochrome p450 family are monooxygenases that metabolize lipophilic procarcinogens, often converting them to highly carcinogenic epoxides in the process (Johnson et al., 1994). Isothiocyanates such as phenethyl isothiocyanate (PEITC), benzyl isothiocyanate (BITC) and sulforaphane modify the balance of Phase I and II xenobiotic metabolizing enzymes that are expressed in liver, and in epithelial cells including those of the colon. Phase II enzymes such as the glutathione transferase family (GST) metabolize the products of Phase I activity to form inactive, water-soluble conjugates that are readily excreted in urine. Hecht and colleagues have studied the effects of PEITC on the induction of lung tumours in a rat model by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), in considerable detail. In one study, lung tumours were induced in 70% of the control rats given only NNK, but only 5% of those treated with both NNK and PEITC (Hecht et al., 1996). In rats given PEITC there was a marked reduction in a blood borne biomarker of NNK activation, 4-hydroxy-1-(3-pyridyl)-1- butanone-releasing hemoglobin adducts, and a significant increase in excretion of two NNK metabolites, (4-(methylnitrosamino)-1-(3- pyridyl)-1-butanol and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronide). Taken together, these findings provide good evidence that PEITC has the potential to block the carcinogenic effects of tobacco smoke.

Recent studies have provided good evidence that glucosinolate breakdown products can modify the risk of cancer in humans. In a cohort study, London et al. (2000) examined the relationship between the concentration of isothiocyanate metabolites in urine and subsequent risk of lung cancer in a group of Chinese men. The subjects were also tested for the presence of deletion-polymorphisms of the *GSTM1* and *GSTT1* genes, which code for different members of the GST enzyme family. During 10 years of follow-up, smokers with detectable levels of isothiocyanate metabolites in urine were at a reduced risk of cancer compared to controls (RR = 0.65 but the protective effect was observable only in subjects with homozygous deletion of *GSTM1* or *GSTT1*, and strongest in those with deletion of both *GSTM1* and *GSTT1* (RR = 0.28; $p<0.01$).

The GST enzymes play a major role in the detoxification of environmental mutagens, but they also metabolise anticarcinogenic phytochemicals, including the isothiocyanates. Loss of *GSTT1* and *GSTM1* would be expected to reduce the efficiency with which tobacco smoke carcinogens are metabolized. Individuals with null genotype for both *GSTM1* and *GSTT1* were at considerably greater risk of lung cancer if they lacked isothiocyanates in their urine (RR = 2.79). However the adverse effect of the reduced metabolic capacity resulting from GST-null status seemed to be outweighed in subjects who showed evidence of a high intake of glucosinolates. This can be explained by the reduced metabolism of isothiocyanates, leading to a more prolonged exposure of target cells to their anticarcinogenic effects. A similar relationship with lung cancer has recently been reported for another Chinese population (Zhao et al., 2001), and the protective effects of broccoli against colorectal polyps have also been reported to be dependent upon GST status (Lin et al., 1998).

The ability of isothiocyanates to induce Phase II enzymes may be linked mechanistically to their recently recognised ability to suppress the proliferation of preneoplastic cells in a number of different experimental systems (Kirlin et al., 1999). The inhibitory effects of isothiocyanates on cell viability have been apparent for a number of years. Their antimicrobial properties first attracted particular interest (Zsolnai, 1971), but more recently it was established that benzyl and phenethyl isothiocyanates inhibited the growth of mammalian cells. Musk et al. showed that allyl isothiocyanate (AITC) (Musk and Johnson, 1993), PEITC and BITC (Musk et al., 1995) caused growth inhibition and a reduction in clonal survival in the human colorectal tumour cell line HT29. When these cells were induced to undergo epithelial differentiation by exposure to sodium butyrate or dimethylformamide, they became significantly more resistant to the cytotoxic effects of isothiocyanates. HeLa cells exposed

to PEITC have been shown to undergo blockade of the cell cycle at G2/M (Hasegawa et al., 1993), or apoptosis (Yu et al., 1998), apparently associated with increased levels of c-Jun N-terminal kinase 1 (Yu et al., 1996). Human leukaemia cells (HL60) and human myeloblastic leukaemia 1 cells have also been reported to undergo caspase-dependent apoptosis following exposure to phenethyl isothiocyanate (Xu and Thornalley, 2000). Overall there is consistent evidence that the isothiocyanates cause disruption to the cell cycle in a variety of cell lines but the precise details of these effects vary from one compound to another. For example, whereas sulforaphane causes a block in cell cycle at G1/S (Gamet-Payrastre et al., 2000) PEITC and allyl AITC cause a block at G2/M (Hasegawa et al., 1993; Lund et al., 2001; Xu and Thornalley, 1999). However PEITC-NAC conjugate appears to block in G1 (Chiao et al., 2000).

The ability of isothiocyanates to induce a complete apoptotic programme *in vitro* is less well established. After exposure to AITC, the human colorectal cell line HT29 becomes blocked in G2/M, and undergoes nuclear condensation and detachment from the substratum. However this process is not caspase-dependent and the floating cells do not exhibit classical markers of apoptosis (Lund et al., 2000). The failure to undergo complete apoptosis may reflect the absence of wild-type *p53* expression in these cells.

As we have seen, there is growing evidence that consumption of brassica vegetables can modulate the activity of Phase II enzyme activity in human beings (Nijhoff et al., 1995a,b; Steinkellner et al., 2000), and thus block the induction of DNA damage by chemical carcinogens. However the growing evidence that isothiocyanates can interfere with cell growth raises the possibility that these compounds may be useful as dietary anticarcinogens, or chemopreventive agents for the suppression of neoplastic lesions after the initial damage to the genome. Although the induction of apoptosis may appear to be a potentially adverse form of cytotoxicity, suppression of apoptosis is an important characteristic of tumour cells. Conversely, selective induction of apoptosis in epithelial cells carrying potentially precancerous genetic damage may slow the growth of lesions, or even cause their regression (Johnson, 2001).

There is some experimental evidence to indicate that isothiocyanates can inhibit the development of colorectal cancer in animal models when given either before, or after, treatment with a carcinogen (Chung et al., 2000). In an *in viv*o study from the author's laboratory, a diet enriched with sinigrin, which is the glucosinolate precursor of allyl isothiocyanate, suppressed mitosis and induced an increased level of apoptosis in the colorectal crypts of rats, 48 h after treatment with the colon carcinogen DMH (Smith et al., 1998). This effect was associated with a significant suppression of aberrant crypt foci, which are thought to be precancerous lesions (Bird, 1995). Importantly, sinigrin had no significant effect on crypt cell kinetics and apoptosis in the colon of control rats that had not been treated with a carcinogen (Smith et al., 1998). In later studies, a juice derived by mechanical disruption of uncooked Brussels sprout tissue was shown to exert effects similar to those of AITC on HT29 cells *in vitro*. Moreover, the same juice given by gavage, markedly increased crypt cell apoptosis after treatment with DMH in a rodent model (Smith et al., 2000). Collectively, these results suggest that both isothiocyanates, and the dietary components through which they can be delivered to target tissues, may exert a selective effect against the growth of colorectal epithelial cells carrying DNA damage *in vivo*. However it has not yet been established that glucosinolate breakdown products derived from the diet can suppress the cell cycle and induce apoptosis in the human colorectal mucosa.

Conclusions

Glucosinolates are present at relatively high concentrations in the diet compared to some other phytochemicals. For example, an individual eating 3–4 portions of broccoli per week, which is the level that appears to confer a protective effect against adenomatous polyps, (Lin et al., 1998), may regularly consume 300-400 mg of glucosinolates. However the level of exposure varies considerably with the commercial variety of vegetable eaten, agronomic conditions, processing techniques and culinary habits. It is clear that different glucosinolate breakdown products exert a variety of effects on enzyme expression, cell proliferation and cell survival. The epidemiological evidence for protective effects of brassica vegetables reflects a considerable variety of exposures to different vegetables and dietary patterns. In these circumstances it would be unwise to emphasise the importance of any particular variety of vegetable, and dietary advice to the public should emphasise the benefits of consuming a wide variety of brassica vegetables in many different culinary forms. The levels of glucosinolates in bras-

sica vegetables can be manipulated by plant breeders, and their presence influences both the palatability and potentially, the nutritional properties of these crops (van Doorn et al., 1998). Both issues need to be considered when breeding new varieties of vegetable. As with synthetic drugs, plant secondary plant metabolites may, in principle, have adverse side-effects as well as beneficial properties, and glucosinolate breakdown products are no exception (Langer et al., 1971). Further research is needed to define the biological activities of the glucosinolate breakdown products in greater detail, so that the balance of benefit, risk and consumer preference can be properly defined.

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