

MINI-REVIEW

Cruciferous Vegetables: Dietary Phytochemicals for Cancer Prevention

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Abstract

Relationships between diet and health have attracted attention for centuries; but links between diet and cancer have been a focus only in recent decades. The consumption of diet-containing carcinogens, including polycyclic aromatic hydrocarbons and heterocyclic amines is most closely correlated with increasing cancer risk. Epidemiological evidence strongly suggests that consumption of dietary phytochemicals found in vegetables and fruit can decrease cancer incidence. Among the various vegetables, broccoli and other cruciferous species appear most closely associated with reduced cancer risk in organs such as the colorectum, lung, prostate and breast. The protecting effects against cancer risk have been attributed, at least partly, due to their comparatively high amounts of glucosinolates, which differentiate them from other vegetables. Glucosinolates, a class of sulphur-containing glycosides, present at substantial amounts in cruciferous vegetables, and their breakdown products such as the isothiocyanates, are believed to be responsible for their health benefits. However, the underlying mechanisms responsible for the chemopreventive effect of these compounds are likely to be manifold, possibly concerning very complex interactions, and thus difficult to fully understand. Therefore, this article provides a brief overview about the mechanism of such compounds involved in modulation of carcinogen metabolising enzyme systems.

Keywords: Cruciferous vegetables - glucosinolates - isothiocyanates - chemopreventive - carcinogen

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Introduction

Prevention and/or protection against chemical carcinogens by phytochemicals present in extensively consumed glucosinolate-containing cruciferous vegetables is of great of interest, as they provide a safe and cost effective means of combating cancer. Extensive epidemiological studies revealed repeatedly an inverse relationship between cruciferous vegetable consumption and the incidence of cancer at a number of sites (Ambrosone et al., 2004; Joseph et al., 2004; Zhao et al., 2007; Lam et al., 2009; Bhattacharya et al., 2010). Isothiocyanates, derived from the hydrolysis of glucosinolates, are believed

to mediate the chemopreventive effect of these vegetables.

Around 120 different naturally-occurring glucosinolates have been recognised so far; however their content varies among different cruciferous vegetables (Fahey et al., 2001). Common glucosinolates include glucoraphanin in broccoli, glucoerucin in rocket salad, glucoraphastin in radish as well as gluconasturtiin in watercress. When cruciferous vegetables are disturbed, for example during chopping or chewing, the enzyme myrosinase (β -thioglucosidase glucohydrolase, EC 3.2.3.1) comes into contact with the glucosinolate leading to the formation of the isothiocyanate (Figure 1); furthermore, intestinal microbial myrosinase can contribute to the generation

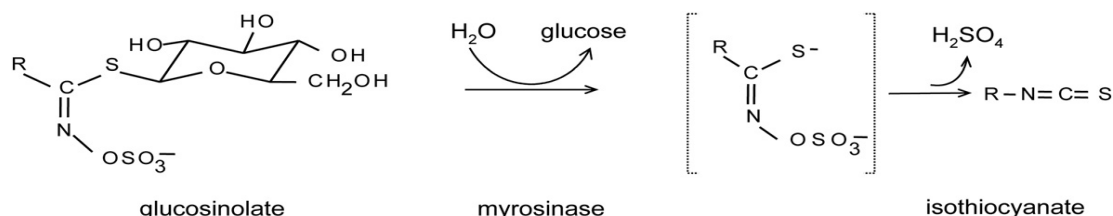


Figure 1. Formation of Isothiocyanates from Glucosinolates. Myrosinase ruptures the β -thioglucoside bond of the glucosinolate molecule to generate a highly unstable aglycone intermediate, followed by non-enzymic intramolecular (Lossen) rearrangement to form isothiocyanate, nitriles, or thiocyanates, depending on the structure of the aglycone, temperature and pH. Adapted from Getahun and Chung (1999); Brown and Hampton (2011)

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of isothiocyanates from their glucosinolate precursors (Getahun and Chung, 1999; Brown and Hampton, 2011).

Eating cruciferous vegetables fully accounts for human dietary exposure to glucosinolates (McGregor et al., 1983). The average intake of glucosinolates is difficult to ascertain because of the variation in plants from diverse cultivars of cruciferous vegetables (Steinbrecher et al., 2009). The consumption of cruciferous vegetables varies between populations. In UK, it was estimated that the average daily intake of total glucosinolates was 14 mg/person/day for a 70 kg individual or 0.2 mg/kg/day (Sones et al., 1984), in contrast to US and Canada, where the average intake was lower (Krul et al., 2002), bearing in mind that some people dislike *Brassica* vegetables whereas others consume large amounts (Holst and Williamson, 2004). As isothiocyanates are most important due to their chemoprevention potential, appreciation of daily intake is valuable for assessing their chemopreventive activity according to dietary guidelines which so far have not been defined.

Chemopreventive mechanisms of isothiocyanates influence all stages of carcinogenesis; nevertheless, impairment of the formation of DNA adducts with chemical carcinogens by limiting the generation of their reactive intermediates via modulation of carcinogen-metabolising enzyme systems is considered to be one of the most important; decreased bioavailability of genotoxic intermediates can be achieved by inhibition of cytochrome P450 bioactivation and/or induction of phase II detoxification enzymes (Robbins et al., 2005; Ioannides et al., 2010).

Possible Role of Intact Glucosinolates in the Chemopreventive Effect of Cruciferous Vegetables

It is generally believed that the chemopreventive activity of cruciferous vegetables is exclusively the result of exposure to degradation products of glucosinolates, which is known as isothiocyanates, and that the parent glucosinolates make no contribution. It has also been assumed that intact glucosinolates would be unable

Table 1. Modulation of Carcinogen-metabolising Enzyme by Intact Glucosinolates in Precision-cut Rat Tissue Slices

System: Glucosinolate	Tissue	Enzyme activity ^{a,b}	
CYP enzymes:			
Glucoraphanin	Liver	EROD↑↑↑	MROD↑↑↑↑
	Lung	EROD↑↑↑↑	
Glucoerucin	Liver	EROD↑↑↑↑	MROD↑↑
	Lung	EROD↑↑↑↑	
Glucoraphasatin	Liver	EROD→	MROD→
Phase II:			
Glucoraphanin	Liver	QR↑, GST↑, EH↑, UGT→	
	Lung	QR→, GST↑	
Glucoerucin	Liver	QR↑, GST↑, EH↑, UGT→	
	Lung	QR→, GST→	
Glucoraphasatin	Liver	QR→, GST→, EH↑, UGT↑	

^a↑small increase; ↑↑modest increase; ↑↑↑marked increase; ↓small decrease; ↓↓modest decrease; ↓↓↓marked decrease; →no effect. ^bEROD, Ethoxyresorufin *O*-deethylase; MROD, methoxyresorufin *O*-dealkylase; QR, quinone reductase; GST, glutathione *S*-transferase; EH, epoxide hydrolase; UGT, glucuronyltransferase

to reach the blood circulation following oral intake. However, at least in rats and dogs, glucosinolates such as glucoraphanin, could be absorbed intact following oral intake (Bheemreddy and Jeffery, 2007; Cwik et al., 2010). Administration of purified glucoraphanin to male F344 rats at 150 μmol/kg led to 5% of the oral dose being found intact in urine but not in the faeces (Bheemreddy and Jeffery, 2007). In addition, glucoraphanin in plasma was detectable in dog and rat treated with glucoraphanin as compared to controls; in dog, plasma levels were in the range of 7.0-34.0 μM after receiving a single oral dose of 500 μmol/kg/day for 3 days, while in rats the mean concentrations determined after 13 days dosing at 30, 115, 230, and 1150 μmol/kg/day were 0.1, 0.5, 1.0 and 4.0 μM, respectively (Cwik et al., 2010); however, these studies employed high doses of glucosinolates in comparison with the average human intake (0.5 μmol/kg/day). Nowadays humans are exposed, however, to higher level of glucosinolates since there are available as supplement pills that are believed to provide a health benefit. In this situation, the glucosinolates are taken in the absence of myrosinase, and only microbial myrosinase in the intestine may catalyse the formation of the isothiocyanate, so that high levels of glucosinolates may be attained sufficient to modulate carcinogen-metabolising enzyme systems. Recent study was performed to assess whether intact glucosinolates have potential chemopreventive activity. Studies were undertaken *in vitro*, employing precision-cut tissue slices of rat liver and lung (Abdull Razis et al., 2010; 2011a; 2011b; 2012a).

Table 1 summarises the effects of three glucosinolates, glucoraphanin, glucoerucin and glucoraphasatin on carcinogen-metabolising enzyme systems in precision-cut rat liver and lung slices. These studies clearly show, for the first time, that in the both tissues, glucoerucin and glucoraphanin increased CYP1 activity (Abdull Razis et al., 2010; 2011b) as exemplified by the *O*-dealkylations of methoxy- and ethoxyresorufin (Namkung et al., 1988). However, although this may be considered detrimental in carcinogen bioactivation, the effect is very modest compared with environmental contaminants such as benzo[a]pyrene, a strong inducer of CYP1 enzymes. Similar effects were observed in lung slices incubated with the same glucosinolates (Abdull Razis et al., 2011b). In contrast, glucoraphasatin failed to show an effect (Abdull Razis et al., 2012c), suggesting that the response may be dependent on the side-chain substituent of the glucosinolate (Zhu and Loft, 2003).

Table 2. Modulation of Phase II Detoxification Enzyme by Isothiocyanates in Precision-cut Rat Tissue Slices

Isothiocyanates	Tissue	Enzyme activity ^{a,b}	
Erucin:	Liver	QR↑↑↑, GST↑↑, EH↑, UGT↑↑↑	
	Lung	QR↑↑↑, GST↑↑	
Sulforaphane:	Liver	QR↑↑↑, GST↑↑, EH→, UGT→	
	Lung	QR↑↑↑, GST↑↑	
Phenethyl isothiocyanates:			
	Liver	EH↑↑↑, UGT↑	

^a↑small increase; ↑↑modest increase; ↑↑↑marked increase; ↓small decrease; ↓↓modest decrease; ↓↓↓marked decrease; →no effect. ^bQR, quinone reductase; GST, glutathione *S*-transferase; EH, epoxide hydrolase; UGT, glucuronyltransferase

Intact glucosinolates could also stimulate phase II detoxification enzymes (Abdull Razis et al., 2010; 2011a; 2011b; 2012c). These studies provide strong evidence that glucosinolates have the potential to modulate enzyme systems involved both in the bioactivation and detoxification of chemical carcinogens, and may play a role in the chemopreventive activity of glucosinolates. However, before such conclusions are reached, it is imperative that the pharmacokinetic behaviour of glucosinolates in humans is investigated so that the *in vitro* data may be extrapolated to the *in vivo* situation.

Most studies carried out on the chemopreventive mechanism of glucosinolates and isothiocyanates were conducted mostly *in vitro*, and the significance of such studies can only be assessed in relationship with attainable plasma/tissue levels. It is customary that the *in vitro* effects are related to the C_{max} , the highest plasma concentration achieved after dietary intake. Inherent to such an approach is the hypothesis that even transient tissue exposure to the biologically-active concentrations is adequate for a complete response in the up-regulation of enzyme systems to be manifested. In precision-cut rat liver slices exposed to glucoraphanin and glucoerucin, for various periods of time up to 24 hours, increase in the *O*-dealkylation of methoxy- and ethoxyresorufin emerged only at incubation periods longer than 6 hours (Abdull Razis et al., 2012a), indicating that for an effective rise in activity of these enzymes, as a result of exposure to glucosinolates, to be manifested, tissue exposure of at least 6 hours to the appropriate concentrations is required.

Modulation of Carcinogen-Metabolising Enzyme Systems by Isothiocyanates

The ability of isothiocyanates to modulate cytochrome P450 enzymes and phase II enzymes such as quinone reductase and glutathione *S*-transferase has been widely established (Yoxall et al., 2005; Hanlon et al., 2008; 2008b; Konsue and Ioannides, 2010a; 2010b; 2010c); however, their effects on other enzyme systems, such as glucuronosyltransferase and epoxide hydrolase, which are also important in carcinogen metabolism, have not attracted much interest, and therefore were addressed in the recent study. As illustrated in Table 2, exposure of rat liver slices to the isothiocyanates, erucin and phenethyl isothiocyanate, elevated glucuronosyltransferase and epoxide hydrolase activities, but not in the case of *R,S*-sulforaphane (Abdull Razis et al., 2011a), commensurate with *in vivo* studies where exposure of rats to sulforaphane failed to increase the activity of this enzyme in the liver (Yoxall et al., 2005). At the mRNA level, however, sulforaphane increased glucuronosyltransferase expression in HepG2 cells (Bacon et al., 2003). On the other hand, consumption of watercress, a major source of phenethyl isothiocyanate, increased the metabolism of nicotine in smokers as a result of increased glucuronidation (Hecht et al., 1999), in concordance with the studies where exposure of rat liver slices to phenethyl isothiocyanate led to an increase in the glucuronidation of 1-naphthol (Abdull Razis et al., 2011a).

Previous *in vitro* studies demonstrated significant

differences in the induction potential of phase II enzymes among individual isothiocyanates that was believed to be associated strongly with the intracellular accumulation of the isothiocyanate, which is influenced by the isothiocyanate structure. The oxidation state of the sulphur has a marked effect on the induction potential (Zhang and Talalay, 1998). In the recent studies, sulforaphane had no effect whereas erucin elevated epoxide hydrolase and glucuronosyltransferase activities (Abdull Razis et al., 2011a). It is relevant to point out that erucin is extensively metabolised to form sulforaphane as a result of the oxidation of its sulphide group and, furthermore, sulforaphane is reduced to form erucin *in vivo*. Such an inter-conversion of sulforaphane and erucin may occur *in vitro* during tissue incubation, and in humans consuming broccoli sprouts or broccoli supplements (Kassahun et al., 1997; Clarke et al., 2011).

There have been a few studies concerned with the concentrations of isothiocyanates in plasma and/or their pharmacokinetic behaviour so as to allow the *in vitro* effects of these compounds to be extrapolated in *in vivo* situation. In a human study (Cramer et al., 2012), participants consumed meals supplemented with broccoli sprouts equivalent to 70 μ mol sulforaphane or glucoraphanin powder equivalent to 120 μ mol sulforaphane, achieved plasma levels of about 0.22 μ M and 0.25 μ M, respectively 3 hours after consumption. In another study, subjects consumed 40 g of broccoli sprouts (150 and 71 μ mol glucoraphanin and glucoerucin, respectively) or 6 supplement pills (121 and 40 μ mol glucoraphanin and glucoerucin, respectively) achieved maximum plasma concentrations of sulforaphane and erucin metabolites at 3 h post consumption (Clarke et al., 2011). Nevertheless, intracellular concentrations of isothiocyanates may be higher than those in the plasma; it has already been demonstrated that intracellular concentration may reach 200-fold higher than extracellular concentration within a 3 h of exposure (Ye and Zhang, 2001; Zhang and Callaway, 2002). Exposure of mouse skin papilloma cells to allyl-, benzyl- and phenethyl isothiocyanates and sulforaphane showed that elevation in glutathione levels and, glutathione *S*-transferase and quinone reductase activities was closely dependent on their intracellular accumulation levels, with sulforaphane being the superior inducer (Ye and Zhang, 2001), similar data were presented in the recent study. It should be emphasised that marked differences have been reported in modulation of carcinogen-metabolising enzymes by isothiocyanates when human liver slices from various donors were employed, demonstrating that inter-individual variation may be a factor influencing the chemopreventive potency of isothiocyanates (Moore et al., 2007; Hanlon; 2009; Konsue, 2010).

Studies on the temporal induction of carcinogen-metabolising enzyme systems by isothiocyanates in precision-cut rat liver slices established that the time of incubation necessitated for the increase in activity to be apparent differs among isothiocyanates, ranging from 2 hours in the case of sulforaphane to 6 hours in the case of phenethyl isothiocyanate (Abdull Razis et al., 2012a), clearly indicating that the nature of the side

chain is essential in the up-regulation of this enzyme by isothiocyanates.

Comparison of Sulforaphane Isomers as Modulators of Enzyme Systems

The chemopreventive activity of sulforaphane was established in animal models of cancer using the commercially available racemate, despite the fact that humans are exposed only to the *R*-enantiomer through the diet. Since a principal mechanism of the chemopreventive activity of sulforaphane is modulation of the carcinogen-metabolising enzyme systems, a study was conducted in precision-cut rat liver and lung slices, and in rat hepatoma FAO cells comparing the ability of *R*- and *S*-sulforaphane to modulate these enzyme systems (Abdull Razis et al., 2011d).

In both *in vitro* systems the naturally-occurring *R*-sulforaphane was more effective than the *S*-isomer in modulating various enzyme systems (Abdull Razis et al., 2011d), implying that chemopreventive activity may be underestimated as the racemate was employed. However, in making such conclusions the assumption is made that the two isomers do not differ in their pharmacokinetic behaviour and metabolism, and such studies are essential before conclusions are drawn. Moreover, it would be informative to compare in *in vitro* studies of intracellular accumulation of the two isomers.

Potential of Daikon Glucosinolates to Act as Chemopreventive Agents Through Modulation of Carcinogen Metabolism

An *in vivo* study was undertaken in rats using a glucosinolate-rich Daikon extract containing glucoraphasatin and substantial amounts of glucoraphenin. A major finding was that the lung was refractive to the effect of the Daikon glucosinolates in comparison with the liver (Abdull Razis et al., 2012b). Similarly, supplementation of the diet of rats with phenethyl isothiocyanate caused a rise in glutathione *S*-transferase and quinone reductase activities in the liver whereas the lung was refractive (Guo et al., 1992; Konsue and Ioannides, 2008).

In the liver, especially at the Low dose simulating human intake, there was a rise in CYP1 activity, a consequence of increased enzyme availability, suggesting that the up-regulation of this enzyme by the Daikon extract may involve increased gene transcription, mRNA, and/or protein stabilisation (Abdull Razis et al., 2012b). The rise in CYP1 activity implies increased production of toxic metabolites or that may be detrimental; however, the rise in CYP1 activity is modest compared with others CYP1 inducers to which humans are frequently exposed. Moreover, a number of detoxification enzymes was also up-regulated, although in some cases higher doses were required (Abdull Razis et al., 2012b). Thus, despite a rise in CYP1 activity the balance of bioactivation/detoxification may become more favourable.

In order to better evaluate the potential of Daikon

glucosinolates as chemopreventive agents, additional *in vitro* studies were conducted in precision-cut rat liver slices employing glucoraphasatin, the principal glucosinolate in Daikon. These studies showed a different picture between *in vitro* and *in vivo* studies in which glucosinolate elevated hepatic dealkylations of methoxy-, ethoxy-, pentoxyresorufin and benzyloxyquinoline, as well as marked induction of glutathione *S*-transferase, quinone reductase and glucuronosyl transferase activities in *in vivo*, in contrast to *in vitro* studies; both systems showed a marked increase in epoxide hydrolase activity (Abdull Razis et al., 2012b; 2012c). However, supplementation of the incubation system with myrosinase to generate the isothiocyanate led to a marked rise in glutathione *S*-transferase, quinone reductase and epoxide hydrolase activities and expression as showed in the *in vivo* studies suggesting that the intact glucosinolate was hydrolysed by myrosinase to isothiocyanate (Abdull Razis et al., 2012c).

Interaction of Isothiocyanates and Glucosinolates with the Ah Receptor

The aryl hydrocarbon (Ah) receptor is a cytosolic transcription factor involved increasingly in many pathophysiological processes, so that antagonists of the Ah receptor imply chemopreventive potency of cruciferous vegetables. It regulates carcinogen-metabolising enzymes, for example the CYP1 family of cytochromes P450 and quinone reductase (Köhle and Bock, 1989; Safe, 2001), which play an essential role in the biotransformation of many chemical carcinogens (Ioannides and Lewis, 2004).

Using the chemically-activated luciferase gene expression (CALUX) assay it was established that phenethyl isothiocyanate, erucin and sulforaphane, are such antagonists (Abdull Razis et al., 2011c; 2012d). These isothiocyanates were poor ligands to the Ah receptor and weak inducers of CYP1A1 mRNA levels when incubated in precision-cut rat liver slices. They effectively antagonised, however, in a non-competitive manner, the activation of the receptor by the avid ligand benzo[a]pyrene. Among the two isomers of sulforaphane, the naturally-occurring *R*-isomer was the more efficient (Abdull Razis et al., 2012d). Furthermore, phenethyl isothiocyanate, erucin and sulforaphane suppressed, in concentration-dependent manner, the benzo[a]pyrene-mediated rise in rat hepatic CYP1A1 mRNA levels in rat slices (Abdull Razis et al., 2011c; 2012d), in concordance with studies reporting that these isothiocyanates antagonise the benzo[a]pyrene-mediated increase in the *O*-deethylation of ethoxyresorufin in both rat and human precision-cut liver slices (Hanlon, 2009a; Konsue, 2010a), as well as in human mammary tumour cell line Mcf7, where sulforaphane inhibited benzo[a]pyrene-mediated CYP1A2 induction (Skupinska et al., 2009). It is possible that the effect of isothiocyanates on the *O*-deethylation of ethoxyresorufin is due, to some extent, mechanism-based inhibition associated with these compounds (Yoxall et al., 2005; Hanlon et al., 2008a). However, it is important to point out that it is the phenobarbital-inducible CYP2B enzymes that catalyse the metabolism of phenethyl

isothiocyanate to the metabolite(s) liable for mechanism-based inhibition, in contrast to benzo[a]pyrene inducible CYP1 enzymes, suggesting that mechanism-based inhibition is unlikely to be the dominant mechanism (Lee, 1996; Goosen et al., 2000; Nakajima et al., 2001; Konsue and Ioannides, 2010c). Thus, it can be inferred that isothiocyanates are effective antagonist of the Ah receptor in rat and human liver, and this potential may contribute to their established chemopreventive activity.

Conclusions

Glucosinolates and their isothiocyanates can modulate the activity of carcinogen-metabolising enzyme systems via deactivation of CYP 450 and bioactivation of Phase II detoxification enzymes, which are likely to impact on the chemopreventive activity linked to cruciferous vegetable consumption.

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