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Chemoprevention of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen

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The aim of this study was to investigate the chemopreventive effects of widely consumed cruciferous vegetables, namely Brussels sprouts and red cabbage towards 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced preneoplastic lesions [liver glutathione-*S*-transferase placental positive (GST-P$^+$) foci and colonic aberrant crypt foci (ACF)]. Male F344 rats were treated with IQ (100 mg/kg bw/g) on 10 alternating days and received drinking water supplemented with Brussels sprouts and red cabbage juices (5% v/v) before and during the carcinogen treatment. From each vegetable two different cultivars were tested. Brussels sprouts reduced the frequency of IQ-induced aberrant foci in both organs (41–52% in the colon and 27–67% in the liver). Also, Brussels sprouts drastically diminished (85–91%) the size of liver GST-P$^+$ foci, but no such effect was seen in the colon. With red cabbage, the size of liver GST-P$^+$ foci was markedly reduced (41–83%) whereas the foci frequency was only moderately decreased (19–50%). No protection was seen in the colon after treatment with red cabbage. Cooking (10 min, 100°C) of the vegetables had no influence on their protective effects. The stronger chemoprotective effects of Brussels sprouts may be due to the fact that the overall glucosinolate contents were substantially (2–3-fold) higher than those of the cabbage cultivars, but it was not possible to attribute the reduction of preneoplastic lesions to specific glucosinolates. The activities of hepatic UDP-glucuronosyltransferase form 2 (UDPGT-2) and cytochrome P4501A2 were increased by both vegetables. The induction effect of Brussels sprouts on the activity of UDPGT-2 was more marked than that of the red cabbage cultivars, suggesting that increased glucuronidation of IQ may account for the reduction of the preneoplastic lesions. Our findings support the assumption that *Brassica* vegetables protect against the carcinogenic effects of heterocyclic amines.

Abbrevations: ACF, aberrant crypt foci; GST-P, glutathione-*S*-transferase placental form; HA, heterocyclic amine; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; ITC, isothiocyanate; UDPGT, UDP glucuronosyltransferase.

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Introduction

Numerous epidemiological studies suggest that consumption of cruciferous vegetables is inversely related with the incidence of various forms of cancer, in particular with colorectal cancer (1–4). One of the risk factors for the development of colon cancer may be exposure to heterocyclic aromatic amines (HAs), which are formed during the cooking of meat and fish (5). Therefore, we hypothesized that the cancer protective properties of cruciferous vegetables might be due to their chemopreventive effects against HAs. Indeed, we found recently that garden cress (*Lepidium sativum*), a cruciferous plant and its constituents reduce the induction of DNA damage and aberrant crypt foci (ACF) formation by 2-amino-3-methyl-3H-imidazo[4,5-*f*]quinoline [IQ, (6)], a member of HA which causes colon and liver tumors in rats (5).

ACF and hepatic glutathione-*S*-transferase (GST-P$^+$) foci are early preneoplastic lesions in colon and liver, respectively, which may give rise to the formation of tumors (7–10). The initiation and further development of carcinogen-induced ACF and GST-P$^+$ could be prevented by dietary constituents (11–13).

The major shortcoming of ACF assay models for the identification of chemoprotective agents against HAs is the low foci yield, which hinders the detection of anticarcinogenic effects (14). In an earlier study we observed an increase in the number of IQ-induced colonic ACF by a modified (high fat–low fibre) diet (15).

The chemopreventive effects of cruciferous vegetables towards dietary carcinogens such as nitrosamines and polycyclic aromatic hydrocarbons have been attributed to isothiocyanates and indoles (16–21), which are formed upon hydrolysis of the parent glucosinolates by the enzyme myrosinase. Any process that damages plant tissues leads to the release of myrosinase. Cooking procedures, however, lead to inactivation of the enzyme (22) thereby reducing the release of breakdown products and the protective properties of the vegetables.

In the present study we investigated if commonly consumed cruciferous vegetables are protective towards IQ. Juices from two cultivars each of Brussels sprouts and red cabbage were added to the drinking water (5%, v/v) of rats before and during treatment with IQ. Four months after the last treatment with the carcinogen, the frequencies of IQ-induced hepatic glutathione-*S*-transferase (GST-P$^+$) foci and colonic aberrant crypt foci (ACF) were determined. In order to assess the effect of cooking on the protection of the vegetables, juices were prepared from raw and cooked vegetables and used in the assay. Moreover, to find out which glucosinolates are responsible for the protective effects, we determined the glucosinolate contents of the different vegetables and tried to correlate the results to the effects seen in the aberrant foci experiments. On the basis of our recent findings with garden cress (6), we hypothesized that the mechanism behind the protective effects of cruciferous plants towards IQ is induction of UDP-glucuronosyltransferase (UDPGT), an important enzyme in the detoxification of HAs

(23–25) that can be induced by dietary constituents (26). To ascertain this assumption, we measured the effects of the different vegetables on the activities of UDPGT isozymes and compared the effects with the results obtained in the chemoprevention studies. Additionally, the impact of the juices on cytochrome P4501A2, an enzyme that catalyses the activation HAs (23), was investigated.

Materials and methods

Chemicals

Methylene blue, bovine serum albumin, chloramphenicol, 4-methyl umbelliferone (4-MU) and methoxyresorufin were supplied by Sigma (St Louis, MO, USA). Bradford's reagent was purchased from Biorad (Munich, Germany). 2-Amino-3-methyl-imidazo[4,5-f]quinoline was purchased from Toronto Research Chemicals (Toronto, Canada). GST pi polyclonal antibody was bought from Novocastra (Newcastle, UK).

Juice preparation

Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* 'Cyrus' and *B.oleracea* L. var. *gemmifera* 'Maximus') and red cabbage (*B.oleracea* L. var. *capitata* subvar. *Rubra* 'Roxy' and *B.oleracea* L. var. *capitata* subvar. *Rubra* 'Reliant') were obtained from Novartis Seeds BV (Enkhuizen, The Netherlands). The vegetables were grown on the field and stored at 4°C in the dark after being harvested. Juices were prepared freshly every day from raw and cooked (100°C, 10 min in 100 ml water) materials. After discarding the outer leaves, the vegetables were cut into small pieces and juiced with a commercial machine (Elin T3232). The juices were diluted 1:20 either with tap water (raw vegetables) or cooking water (cooked vegetables). On the basis of preliminary experiments in which the palatability of the juices had been investigated, a 5% (v/v) concentration in the drinking water was chosen for the main experiments.

Analyses of glucosinolates

The glucosinolate contents of the vegetables were determined according to Wathelet *et al.* (27) and Spinks *et al.* (28) with slight modifications. Fresh plant tissue was extracted in boiling methanol (70%) in a water bath at 70°C for 20 min. Subsequently, the extract was centrifuged (1000 *g*, 10 min) and the supernatant collected. The pellet was re-extracted twice following the same procedure. Aliquots of the supernatants were loaded onto ion-exchange mini-columns (DEAE Sephadex A-25) and the glucosinolates were desulphated on-column as described by Helboe *et al.* (29). The desulphoglucosinolates were eluted with water and separated by gradient system high performance liquid chromatography (Thermo Separation Products) using a Nova Pak C18 (5 mm) reverse phase column (3.9 × 159 mm; Waters Corporation, USA). The solvent programme consisted of water for 1 min and a linear gradient over 20 min to water/acetonitrile 80/20 (flow 1.0 ml/min). The desulphoglucosinolates were monitored by UV-absorption at 229 nm and quantified against the internal standard glucotropaeolin. Identification of the individual glucosinolates was done by comparing retention times with pure standards and with a standard rapeseed reference material (BCR 367, Commission of the European Community Bureau of References, Brussels, Belgium).

Animals and treatment

All experiments were carried out with 3-week-old male Fischer 344 rats (body weight, 133–157 g). The animals were purchased from Charles River Inc. (Borchen, Germany) and housed in groups of three in plastic cages under standard conditions (24 ± 1°C, humidity 50 ± 5%, 12 h light/dark cycle).

After 1 week of acclimatization, rats were switched from Purina rat chow (Soest, Germany) to a modified (high fat–fibre free) AIN 76 diet (SDS, Witham, UK) on which they remained for the duration of the experiment. The rats were randomized into 10 groups (eight animals/group), namely (i) negative control; (ii) IQ control; and (iii) combined treatment groups (IQ and vegetables). The juices were given to the rats 5 days before and during the treatment with IQ, which was administered by gavage on 10 alternate days in corn oil (100 mg/kg bw/day). The control group received corn oil and pure tab water. Sixteen weeks after the last IQ treatment, the animals were killed by CO_2 asphyxiation.

Determination of preneoplastic lesions in colon and liver

The livers of the killed animals were weighed and sections fixed in freshly prepared Carnoy's solution and processed as described (30). GST-P$^+$ foci (⩾3 cells) were identified by anti-placental GST stain under a light microscope. Their numbers were calculated per cm^2 evaluated tissue section and at least 1 cm^2 per animal was evaluated with an automatic image analyser (Lucia, Nikon; Meerbusch, Germany).

For the determination of ACF, the colon was removed and cleaned with Ringer's solution, then cut open along the longitudinal median and fixed flat in 10% buffered formalin (pH 7.5) for 24 h. The samples were stained in 0.2% methylene blue and the number of ACF/colon and the number of crypts/focus were evaluated for the entire length of the colon from each rat at a 60-fold magnification as described by Bird (31).

Enzyme measurements

The effects of the vegetables on enzyme activities were monitored in a separate experiment. The rats (4/group) received for 1 week 5% (v/v) fresh juices of raw and cooked plant material. On the eighth day, the animals were killed and liver and colon microsomes prepared according to Ryand *et al.* (32). Protein levels of the microsomes were determined by the method of Bradford (33). UDPGT-1 and UDPGT-2 activities were measured by using 4-methyl umbelliferone and chloramphenicol as substrates according to Lilienblum *et al.* (34) and Young and Lietmann (35) with slight modifications, respectively. Cytochrome P4501A2 activity was determined using methoxyresorufin as a substrate according to Lubet *et al.* (36) at an excitation of 550 nm and an emission wavelength of 585 nm.

Statistical analysis

The numbers of ACF as well as the number and area of GST-P$^+$ foci were compared using ANOVA and linear contrast after homogenizing the variance using logarithmic transformation. Induction of P4501A2 and UDPGT-2 activity was analysed with ANOVA following Dunnet's multiple comparison test.

Results

Glucosinolate content of the vegetables

The overall glucosinolate content of Brussels sprouts cultivars was 2–3-fold higher than those of the red cabbage varieties (Table I). The level of the different glucosinolates varied widely not only between the different vegetables but also between the different cultivars of the same vegetable. Sinigrin dominated in Brussels sprouts 'Cyrus' (68% of the total glucosinolate content) whereas 'Maximus' was additionally rich in iberin. In the red cabbage cultivars, sinigrin and glucoraphanin were the most abundant glucosinolates in 'Roxy' and 'Reliant', respectively.

Effect of treatment on body weight and relative liver weight

Table II shows that none of the treatments had an influence on either body weight or liver weight of the animals. Also, supplementation of the drinking water with juices had no significant effect on food consumption.

Effect of the vegetables on hepatic GST-P$^+$ foci

All vegetables caused a reduction in the number and size of hepatic GST-P$^+$ foci but the effects of the different cultivars varied over a broad range (Table III). Brussels sprouts were consistently more effective than red cabbage. The strongest effect was measured with raw 'Cyrus', which reduced the number and size of foci by 67% and 91%, respectively. Interestingly, all juices caused a more pronounced reduction in the size than in the frequency of the foci. No significant differences were found between the protective properties of juices from cooked and raw vegetables.

Effect of the vegetables on colonic aberrant crypt foci

IQ caused a marked induction of ACF (Table IV). The majority of these lesions was located in the distal part of the colon (Figure 1). Both cultivars of Brussels sprouts decreased the number of IQ-induced ACF markedly, and the extent of reduction was similar (~50%) in all parts of the colon. With the red cabbage cultivars, only a slight, statistically non-significant reduction of the foci number was found. In contrast to the findings in the liver, the crypt multiplicity, which reflects the size of the foci was not decreased by any of the vegetables (Table IV).

Table I. Glucosinolate content of Brussels sprouts ('Cyrus' and 'Maximus') and red cabbage ('Roxy' and 'Reliant')[a]

Glucosinolates	Brussels sprouts Cyrus	Brussels sprouts Maximus	Red cabbage Roxy	Red cabbage Reliant
Iberin	4.4[b]	10.8	1.13	0.7
Progoitrin	3.3	2.7	0.54	0.8
Sinigrin	22.7	9.1	5.16	0.7
Glucoraphanin	0.4	3.0	0.75	5.0
Gluconapin	3.0	2.0	2.20	0.7
4-OH-Glucobrassicin	0.2	0.3	0.65	0.6
Glucobrassicin	3.2	2.9	0.47	2.1
4-Meglucobrassicin	0.8	0.7	0.33	0.2
Neoglucobrassicin	0.0	0	0.05	0.0
Total glucosinolate content	38.0	31.5	11.28	12.0

[a]Fresh plant tissue was extracted with methanol, the extract centrifuged and the resulting supernatant desulphated. Subsequently, the desulphoglucosinolates were separated by high performance liquid chromatography. Glucotropaeolin was used as an internal standard. Identification of the individual glucosinolates was done by comparing the retention times with pure standards and with a standard rapeseed reference material.
[b]Values represent glucosinolate contents in μmol/g dry weight.

Table II. Consumption of Brussels sprouts and red cabbage juices, body weight gain of rats and relative liver weight

Treatment group	Consumption of juice-supplemented water/rat/day[a] (ml)	Initial/final body weight[b] (g)	Relative liver weight[b] (g)
Neg. control	17.5 ± 3.4	135.0 ± 7.2/290.5 ± 11.9	2.7 ± 0.2
IQ	18.5 ± 4.0	136.9 ± 12.1/299.9 ± 25.6	2.8 ± 0.1
Red cabbage			
IQ + Roxy, raw	14.0 ± 0.9	133.8 ± 11.8/292.5 ± 21.0	2.9 ± 0.3
IQ + Roxy, cooked	14.8 ± 1.9	134.8 ± 9.2/301.4 ± 20.9	2.8 ± 0.3
IQ + Reliant, raw	13.7 ± 2.2	130.8 ± 12.7/287.3 ± 19.2	2.7 ± 0.2
IQ + Reliant, cooked	14.2 ± 1.5	122.4 ± 10.2/295.3 ± 11.5	2.8 ± 0.1
Brussels sprouts			
IQ + Cyrus, raw	13.5 ± 2.0	124.6 ± 8.1/291.7 ± 18.1	2.7 ± 0.2
IQ + Cyrus, cooked	15.7 ± 2.8	136.5 ± 6.7/288.2 ± 20.1	2.8 ± 0.1
IQ + Maximus, raw	13.3 ± 1.0	130.3 ± 14.8/301.1 ± 33.8	2.8 ± 0.2
IQ + Maximus, cooked	15.7 ± 1.3	133.0 ± 7.3/298.6 ± 19.2	2.7 ± 0.2

[a]The juices were prepared fresh every day and diluted 1:20 either with tap water or cooking water (in the experiment with cooked vegetables). The consumption of water was measured daily during the entire supplementation period. The negative control and IQ groups received tap water. Values indicated are means ± standard deviations of eight animals per group.
[b]The body weights of the animals were measured every week. The relative liver weights were calculated on the basis of the body weights of the animals on the day of killing. Values indicated are means ± standard deviation of eight animals per group.

Table III. Influence of red cabbage ('Roxy' and 'Reliant' varieties) and Brussels sprouts ('Cyrus' and 'Maximus' varieties) juices on IQ-induced liver GST-P$^+$ foci in the F344 rat[a]

Experimental group	Number of foci/cm^2	Inhibition (% of IQ)	Total area (mm^2/cm^2)	Inhibition (% of IQ)
Neg. control	0.10 ± 0.27	–	0.0001 ± 0.0003	0
IQ	13.00 ± 4.62	0	0.0903 ± 0.0931	
Red cabbage				
IQ + Roxy, raw	10.26 ± 3.01	21	0.0223 ± 0.0138[b]	75
IQ + Roxy, cooked	7.47 ± 3.75[b]	43	0.0166 ± 0.0009[b]	82
IQ + Reliant, raw	6.52 ± 3.99[b]	50	0.0155 ± 0.0121[b]	83
IQ + Reliant, cooked	10.51 ± 6.19	19	0.0530 ± 0.0479	41
Brussels sprouts				
IQ + Cyrus, raw	4.32 ± 1.63[b]	67	0.0079 ± 0.0062[b]	91
IQ + Cyrus, cooked	7.01 ± 4.10[b]	46	0.0105 ± 0.0061[b]	88
IQ + Maximus, raw	9.45 ± 2.29	27	0.0137 ± 0.0053[b]	85
IQ + Maximus, cooked	5.38 ± 2.29[b]	59	0.0078 ± 0.0053[b]	91

[a]Rats were given a 5% (v/v) juice of Brussels sprouts or red cabbage in the drinking water 5 days before and during the IQ treatment (100 mg/kg body weight, p.o. on 10 alternating days). 16 weeks after the last IQ treatment, the animals were killed and the livers removed. Subsequently, liver tissue sections were stained and the frequency and size of GST-P$^+$ foci registered. Data are means ± SD from eight animals/group.
[b]Statistically significant relative to IQ controls as determined by analysis of variance ($P < 0.05$).

Table IV. Influence of red cabbage ('Roxy' and 'Reliant') and Brussels sprouts ('Cyrus' and 'Maximus') juices on IQ-induced colonic ACF frequency and crypt multiplicity in F344 rats[a]

Experimental group	ACF/rat	Inhibition (% of IQ)	Aberrant crypt/ACF	Inhibition (% of IQ)
Neg. control	0.00	–		–
IQ	10.33 ± 3.87	0	1.45 ± 0.24	0
Red cabbage				
IQ + Roxy, raw	8.25 ± 4.95	20	1.33 ± 0.26	8
IQ + Roxy, cooked	9.66 ± 3.06	16	1.46 ± 0.16	0
IQ + Reliant, raw	8.25 ± 3.72	20	1.31 ± 0.18	10
IQ + Reliant, cooked	8.57 ± 5.06	17	1.42 ± 0.28	2
Brussels sprouts				
IQ + Cyrus, raw	5.50 ± 2.16[b]	47	1.39 ± 0.39	4
IQ + Cyrus, cooked	5.00 ± 1.92[b]	52	1.38 ± 0.23	5
IQ + Maximus, raw	6.00 ± 4.10[b]	42	1.55 ± 0.40	0
IQ + Maximus, cooked	6.14 ± 2.19[b]	41	1.43 ± 0.25	1

[a]The animals were treated with IQ and the juices as described in the footnote of Table III. 16 weeks after the last IQ treatment, the animals were killed, colons removed and the number of ACF and crypts/focus evaluated. Data are means ± SD from eight animals/group.
[b]Statistically significant relative to IQ controls as determined by analysis of variance ($P < 0.05$).

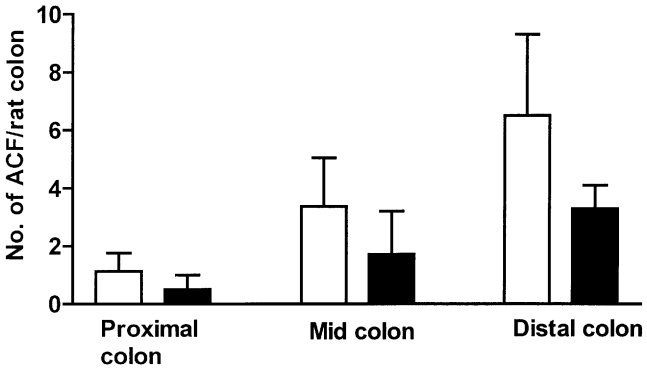

Fig. 1. Distribution of ACF in the colon of rats treated with either IQ alone or IQ and cooked Brussels sprouts 'Cyrus'. The colon was removed intact by severing at the caeco-colonic and colonic-rectal junctions and processed as described in the Materials and methods section. The sections designated as proximal, middle and distal part of the colon represent the first, second and third fraction of the total length.

Induction of cytochrome P450 1A2 and UDPGT

The effects of the juices on the activity of hepatic cytochrome P4501A2 and UDPGT-2 are summarized in Table V. Raw and cooked 'Maximus', raw 'Cyrus' and raw 'Reliant' increased the activity of hepatic UDPGT-2 significantly, whereas only raw and cooked 'Maximus' increased the activity of hepatic P4501A2. None of the vegetables modified the activity of hepatic UDPGT-1 (data not shown). The activities of UDPGT 1 and UDPGT 2 in the colon were ⩾90% lower than those measured in the liver and there were no significant differences between the control and treatment groups (data not shown).

Discussion

According to our knowledge this is the first report on the prevention of HA-induced preneoplastic lesions by commonly consumed cruciferous vegetables. These observations support our earlier assumption, which was based on the results obtained with garden cress (6), that cruciferous plants are protective towards the carcinogenic effects of cooked food mutagens.

By using a modified diet composed of high fat but without fibre, it was possible to study the prevention of IQ-induced preneoplastic lesions simultaneously in liver and colon, the main target organs for tumor induction by HAs of the aminoimidazoazarene group.

All four cultivars reduced the size and number of GST$^+$ foci in the liver, albeit to a different extent. The maximum reduction in the foci frequency was around 50%, whereas the foci size was decreased by up to 90% (Table III). These findings indicate that the vegetables prevent not only the formation of initiated cells but also their clonal expansion. Earlier studies indicated that *Brassica* vegetables and their constituents inhibit the induction of hepatic preneoplastic foci by aflatoxin and diethylnitrosamine (37–39) and Hirose and his coworkers (40) were the first who reported that phenetyl-ITC, a breakdown product of gluconastruitiin found in some cruciferous plants, reduces the number of hepatic GST$^+$ foci induced by a HA (2-amino-6-methyldipyrido[1,2-a:3,2-d]imidazole) in rats.

In the colon, a different pattern of protection was seen as compared to that observed in the liver (Table IV) in that Brussels sprouts but not red cabbage were protective and none of the juices reduced the multiplicity of the crypts, which reflects the size of the foci. This observation suggests that the vegetables prevent only the formation but not the development of the preneoplastic lesions in this organ. At present, although a few studies on the protective effects of specific *Brassica* constituents towards HA-induced ACF are available (41,42), no data concerning chemoprevention by *Brassica* vegetables are available.

Our findings indicate that the chemoprotective properties of Brussels sprouts and red cabbage involve inhibition of the formation (in both liver and colon) as well as development (only in liver) of preneoplastic lesions. One of the most important mechanisms of chemoprotection is induction of phase II enzymes, which detoxify DNA-reactive metabolites and thereby inhibit the formation of initiated cells (43,44). It has been postulated by Sparnins and Wattenberg (45) two decades ago that induction of glutathione *S*-transferase (GST) by constituents of *Brassica* vegetables and other dietary constituents accounts for their anticarcinogenic effects towards polycyclic aromatic hydrocarbons. However, the role of this enzyme in the detoxification of HAs is unclear (23). In previous experiments with garden cress we failed to detect an induction of GST, but the reduction of IQ-induced ACF formation was

Table V. Effect of red cabbage ('Roxy' and 'Reliant' varieties) and Brussels sprouts ('Cyrus' and 'Maximus' varieties) juices on the activities of hepatic cytochrome P4501A2 and UDPGT-2 in F344 rats[a]

Experimental group	Cytochrome P450 1A2 (pmol/mg/min)	Induction (% of control)	UDPGT-2 (pmol/mg/min)	Induction (% of control)
Neg. control	0.54 ± 0.28	100	185.86 ± 18.79	100
Red cabbage				
IQ + Roxy, raw	0.99 ± 0.37	183	211.16 ± 7.67	114
IQ + Roxy, cooked	0.97 ± 0.54	180	230.20 ± 35.86	124
IQ + Reliant, raw	0.60 ± 0.01	111	295.29 ± 14.95[c]	159
IQ + Reliant, cooked	1.12 ± 0.16	207	224.46 ± 29.53	121
Brussels sprouts				
IQ + Cyrus, raw	1.29 ± 0.13	239	351.79 ± 16.08[c]	189
IQ + Cyrus cooked	nd[d]	nd[d]		
IQ + Maximus, raw	1.41 ± 0.41[c]	261	412.79 ± 60.11[c]	222
IQ + Maximus, cooked	1.54 ± 0.45[c]	285	342.05 ± 12.25[c]	184

[a]Rats received 5% (v/v) red cabbage or Brussels sprouts juice in the drinking water for 7 days. On the eighth day, the animals were killed, liver microsomes prepared and activities of cytochrome P4501 A2 and UDPGT-2 measured using methoxyresorufin and chloramphenicol, as substrates. Data are means ± SD per group.
[b]The percentage induction in Brussels sprouts and red cabbage juices-treated groups is relative to negative controls.
[c]Statistically significant relative to negative controls as determined by Wilcoxon's matched pair test ($P < 0.05$).
[d]nd: not determined.

paralleled by a pronounced induction of UDPGT form 2 (6). Therefore, we hypothesized that the enhancement of the activity of this enzyme might be causally related to the protective effects. Indeed, the results of the present experiments support this assumption. Both red cabbage and Brussels sprouts enhanced the activity of UDPGT-2 (Table V) but the effect of the latter was stronger than the former. Human and rat UDGPT isozymes were reported to play an important role in the detoxification of HAs (25,46) and the activity of these enzymes is known to be induced by cooked Brussels sprouts (47) and glucosinolates (26). *Brassica* vegetables can also induce glucuronidation reactions in humans. Pantuck *et al.* (48) reported an increase in the urinary excretion of paracetamol glucuronides in man after consumption of Brussels sprouts and cabbage. Also, recently, Knize and his cowokers (49) reported that consumption of broccoli increases the excretion of glucuronidation products of PhIP. Another possible mechanism of protection is the inhibition of enzymes which are involved in the activation of HAs. On the basis of results of *in vitro* experiments with bacteria, it was postulated that the antimutagenic effects of juices of *Brassicas* and other vegetables against IQ might involve inhibition of cytochrome P450 isozymes (50,51). An identical mode of action was postulated by Barcelo *et al.* (52) who found pronounced inhibition of IQ-induced mutagenicity by sulforaphane in a cell line expressing human CYP1A. The results of the present study, however, show that under *in vivo* conditions no such inhibitory effects take place. In contrast, even a moderate induction of MROD, a marker of P4501A2 which plays a key role in the activation of IQ and other HAs (23) was observed with some of the juices (Table V). This latter finding is in agreement with the result of an earlier investigation (53).

The suppression of preneoplastic foci development by the vegetables may be described to induction of apoptosis. A number of recent articles report induction of apoptosis by constituents of cruciferous vegetables *in vitro* (54–56) and *in vivo* (57). In a study by Smith *et al.* (12), the reduction of dimethylhydrazine-induced ACF in rats was reduced by sinigrin, one of the most abundant glucosinolates in *Brassica* vegetables, and was paralleled by a strong increase in the apoptosis rate. Therefore, the authors postulated that inhibition of ACF formation is due to a selective deletion of damaged stem cells in the crypts through apoptosis.

The chemical analysis of the vegetables shows that the level of individual glucosinolates differ strongly, even between cultivars of the same vegetable (Table I). Nevertheless, no pronounced differences were seen in the biological effects of different cultivars. Thus, neither the inhibition of preneoplastic lesions nor alterations of the enzymatic activities appear to depend on the level of individual glucosinolates. Rather the overall glucosinolate content, which was substantially (2–3-fold) higher in the Brussels sprouts than in red cabbage, seems to play a more important role in regard to chemoprotection towards IQ and enzyme induction. This assumption is supported by the report of Nho and Jeffery (58) in which synergistic upregulation of different phase II enzymes was found when a combination of glucosinolate derivatives was used.

The stability of glucosinolate degradation products, constituents believed to be responsible for the chemoprotective effect of cruciferous vegetables (16–21), has been addressed in earlier reports. de Vos and Blijleven (59) have found that alkyl or alkenyl isothiocynanates are volatile except those bearing a sulfoxyl terminal side chain and indole moiety such as 3-methylsulfinylpropyl isothiocyanate (iberin isothiocyanate) and 3-indolylyacetonitrile. Sulforaphane (4-methylsulfinylbutyl isothiocyanate) was reported to be a more stable and less volatile compound during processing and cooking (60) whereas allyl isothiocyanate was very volatile although it can also be further decomposed to non-volatile compounds under cooking conditions (61). In a preliminary experiment in which we analysed juices prepared from raw Brussels sprouts for breakdown products, we found iberin, sulforaphane, goitrin and indole-3-carbinol (Verkerk *et al.*, in preparation).

In spite of the fact that the plant enzyme myrosinase, which catalyses the formation of isothiocyanates and indoles from glucosinolates is inactivated by heating, we found no marked difference between the protective effects of cooked and raw vegetables. This might be due to the formation of anticarcinogenic breakdown products of glucosinolates in the gastrointesti-

nal tract by representatives of the intestinal flora (21) and/or by hydrolysis under acidic conditions in the stomach (57).

In many *in vivo* chemoprevention studies with HAs, including those with constituents of *Brassica* vegetables, the doses of putative protective compounds used were by far higher than the levels present in the human diet (58,59). In the present study, the amounts of juice which caused protective effects were quite small (0.6–0.7 ml/day/rat, body weight 250 g) and correspond to consumption of 180–210 ml juice/day/person (body weight 75 kg). The same amount of bioactive juice is contained in regular size vegetable meals (300–400 g). This comparison supports the assumption that *Brassica* vegetables may protect humans against the carcinogenic risks of HAs. The inverse relationship between the incidence of colon cancer and consumption of *Brassica* vegetables, which has been observed in a number of epidemiological studies (1–4), may be due to these effects.

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References

1. Steinmetz,K.A. and Potter,J.D. (1991) Vegetables, fruits and cancer. I Epidemiology. *Cancer Causes Control*, **2**, 325–357.
2. Steinmetz,K.A. and Potter,J.D. (1991) Vegetables, fruits and cancer. II Mechanisms. *Cancer Causes Control*, **2**, 427–442.
3. Trock,B., Lanza,E. and Greenwald,P. (1990) Dietary fibre, vegetables and colon cancer: critical review and meta analyses of the epidemiological evidence. *J. Natl Cancer Inst*., **82**, 650–651.
4. Block,G., Patterson,B. and Subar,A. (1992) Fruit, vegetables and cancer prevention. A review of epidemiological evidence. *Nutr. Cancer*, **18**, 1–29.
5. IARC (1993) Some naturally occurring substances: food items and constituents. In *Heterocyclic Amines and Mycotoxins*, *Vol. 56*. WHO, Lyon.
6. Kassie,F., Rabot,S., Uhl,M., Huber,W., Qin,H.M., Helma,C., Schulte-Hermann,R. and Knasmüller,S. (2002) Chemopreventive effects of garden cress (*Lepidium sativum*) and its constituents towards 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. *Carcinogenesis*, **23**, 1155–1161.
7. Sato,K. (1989) Glutathione transferases as markers of preneoplasia and neoplasia. In Vande Woude,G.F. and Klein,G. (eds) *Advances in Cancer Research*. Academic Press, San Diego, pp. 205–255.
8. Satoh,K., Kitahara,A., Soma,Y., Inaba,Y., Hatayama,I. and Sato,K. (1985) Purification, induction and distribution of placental glutathione S-transferase: a new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis. *Proc. Natl Acad. Sci. USA*, **82**, 3964–3968.
9. Bird,R.P. (1995) Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. *Cancer Lett*., **93**, 55–71.
10. McLellan,E.A. and Bird,R.P. (1988) Aberrant crypts: potential preneoplastic lesions in the murine colon. *Cancer Res*., **48**, 6187–6192.
11. Grasl-Kraupp,B., Bursch,W., Ruttkay-Nedecky,B., Wagner,A., Lauer,B. and Schulte-Hermann,R. (1994) Food restriction eliminates preneoplastic cells through apoptosis and antagonizes carcinogenesis in rat liver. *Proc. Natl Acad. Sci*., **91**, 9995–9999.
12. Smith,T.K., Lund,T.K. and Johnson,T. (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate siningrin. *Carcinogenesis*, **19**, 267–273.
13. Morishita,Y., Yoshimi,N., Kawabata,K., Matsunaga,K., Sugie,S., Tanaka,T. and Mori,H. (1997) Regressive effects of various chemopreventive agents on azoxymethane-induced aberrant crypt foci in the rat colon. *Jpn. J. Cancer Res*., **88**, 815–820.
14. Kassie,F., Mersch Sundermann,V., Matsunaga,K., *et al.* (2002) Development and application of test methods for the detection of dietary constituents which protect against heterocyclic aromatic amines. *Mutat. Res.* (in press).
15. Uhl,M., Darroudi,F., Seybel,A., Steinkellner,H., Kassie,F., Grasl-Kraupp,B., Mersch-Sundermann,V. and Knasmüller,S.(2001) Development and use of new experimental models for the identification of DNA-protective and anticarcinogenic dietary plant constituents. In Kreft,I. and Skrabanja,V. (eds) *Molecular and Genetic Interactions Involving Phytochemicals.* COST, Ljubljana, pp. 21–33.
16. Wattenberg,L.W. (1974) Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulphur containing compounds. *J. Natl Cancer Inst*., **52**, 1583–1587.
17. Wattenberg,L.W. (1977) Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J. Natl Cancer Inst*., **58**, 395–398.
18. Wattenberg,L.W. (1983) Inhibition of neoplasia by minor dietary constituents. *Cancer Res*., **43**, 2448s–2551s.
19. Chung,F.L., Wang,M. and Hecht,S.S. (1985) Effect of dietary indoles and isothiocyanates on N-nitrosodimethylamine and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone a-hydroxylation and DNA methylation in rat liver. *Carcinogenesis*, **6**, 539–543.
20. Morse,M.A., Amin,S.G., Hecht,S.S. and Chung,F.L. (1989) Effect of aromatic isothiocyanates on tumorigenicity, O^6-methyl guanine formation and metabolism of the tobacco specific nitrosamine 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Cancer Res*., **49**, 2894–2897.
21. Zhang,Y. and Talalay,P. (1994) Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Res*., **54**, 1976s–1981s.
22. Fenwick,G.R., Heaney,R.K. and Mullin,W.J. (1983) Glucosinolates and their breakdown products in food and food plants. *CRC Crit. Rev. Food Sci. Nutr*., **18**, 123–201.
23. King,R.S., Kadlubar,F.F. and Turesky,R.J. (2000) In vivo metabolism. In Nagao,M. and Sugimura,T. (eds) *Food Borne Carcinogens*. Wiley, New York, pp. 90–111.
24. Kaderlik,K.R., Mulder,G.J., Turesky,R.J., Lang,N.P., Teitel,C.H., Chiarelli,M.P. and Kadlubar,F.F. (1994) Glucuronidation of *N*-hydroxy heterocyclic amines by human and rat liver microsomes. *Carcinogenesis*, **15**, 1695–1701.
25. Nowell,S.S., Massengill,J.S., Williams,S., *et al.* (1999) Glucuronidation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by human microsomal UDP-glucuronosyltransferases: identification of specific UGT1A family isoforms involved. *Carcinogenesis*, **20**, 1107–1114.
26. Nugon-Baudon,L., Rabot,S., Szylit,O. and Raibaud,P. (1990) Glucosinolate toxicity in growing rats: interactions with the hepatic detoxification system. *Xenobiotica*, **20**, 223–230.
27. Wathelet,J.P., Wagstaffe,P.J. and Boeke,A. (1991) The certification of the total glucosinolate and sulfur contents of three rapeseeds (colza*). Commission Euro. Commun.*, EUR 13339 EN, 67–70.
28. Spinks,A., Sones,K. and Fenwick,G.R. (1984) The quantitative analysis of glucosinolates in cruciferous vegetables, oil seeds and forage crops using high performance liquid chromatography. *Fette Seifen Anstrichmittel.*, **86**, 228–231.
29. Helboe,P., Olson,O. and Sorensen,H. (1980) Separation of glucosinolates by HPLC. *J. Chromatogr.*, **197**, 199–205.
30. Grasl-Kraupp,B., Luebeck,G., Wagner,A., Löw-Baselli,A., de Gunst,M., Waldhör,T., Moolgavkar,S. and Schulte-Hermann,R. (2000) Quantitative analysis of tumor initiation in rat liver: role of cell replication and cell death (apoptosis). *Carcinogenesis*, **21**, 1411–1421.
31. Bird,R.P. (1987) Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. *Cancer Lett*., **37**, 147–151.
32. Ryand,L., Lu,A. and Levin,W. (1978) Purification of cytochrome P-450 and P-448 from rat liver microsomes. In Fleisher,S. and Packer,L. (eds) *Methods in Enzymology.* Academic Press, New York, pp. 113–123.
33. Bradford,M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**, 248–254.
34. Lilienblum,W., Walli,A.K. and Bock,K.W. (1982) Differential induction of rat liver microsomal UDP-glucuronosyltransferase activities by various inducing agents. *Biochem. Pharmacol*., **31**, 907–913.
35. Young,W.S. and Lietman,P.S. (1978) Chloramphenicol glucuronosyltransferase: assay, ontogeny and inducibility. *J. Pharmacol. Exp. Ther*., **204**, 203–211.
36. Lubet,R.A., Mayer,R.T., Cameron,J.W., Nims,R.W., Burke,M.D., Wolff,T. and Guengerich,F.D. (1985) Dealkylation of pentoxyresorufin: a rapid and sensitive assay for measuring induction of cytochrome (s) P-450 by pentobarbital and other xenobiotics in the rat. *Arch. Biochem. Biophys*., **238**, 43–48.
37. Boyd,J.N., Babish,N.G. and Stoewsand,G.S. (1982) Modification of beet and cabbage diets of aflatoxin B_1-induced rat plasma alpha-foetoprotein elevation, hepatic tumorigenesis, and mutagenicity of urine. *Food Chem. Toxicol*., **20**, 7–52.
38. Jang,J.J., Cho,K.J., Lee,Y.S. and Bae,J.H. (1991) Modifying responses of allyl sulfide, indole-3-carbinol and germanium in a rat multi-organ carcinogenesis model. *Carcinogenesis*, **12**, 691–695.

39. Tanaka,T., Mori,Y., Morishita,Y., Hara,A., Ohno,T., Kojima,T. and Mori,H. (1990) Inhibitory effect of sinigrin and indole-3-carbinol on diethylnitrosamine-induced hepatocarcinogenesis in male ACI/N rats. *Carcinogenesis*, **11**, 103–106.
40. Hirose,M., Hasegawa,R., Kimura,J., *et al.* (1995) Inhibitory effects of 1-*O*-hexyl-2,3,5-trimethlylhydroquinone (HTHQ), green tea catechins and other antioxidants on 2-amino-6-methyldipyridol[1,2-*a:*3,2-*d*]imidazole (Glu-P1)-induced rat hepatocarcinogenesis and dose-dependent inhibition by HTHQ of lesion induction by Glu-P-1 or 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx). *Carcinogenesis*, **16**, 3049–3055.
41. Xu,M., Bailey,A.C., Hernaez,J.F., Taoka,C.R., Schut,H.A.J. and Dashwood,R.H. (1996) Protection by green tea, and indole-3-carbinol against 2-amino-3-methylimidazo[4,5-*f*]quinoline-induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinogenesis*, **17**, 129–134.
42. Guo,D., Schut,H.A.J., Davis,C.D., Snyderwine,E.G., Bailey,G.S. and Dashwood,R.H. (1995) Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced DNA adducts and colonic aberrant crypts in the F344 rat. *Carcinogenesis*, **16**, 2931–2937.
43. De Flora,S. and Ramel,C. (1988) Mechanisms of inhibitors of mutagenesis and carcinogenesis. Classification and overview. *Mutat. Res.*, **202**, 285–306.
44. De Flora,S. and Ramel,C. (1990) Classification of mechanisms of inhibitors of mutagenesis and carcinogenesis. *Basic Life Sci*., **52**, 461–462.
45. Sparnins,V.L. and Wattenberg,L.W. (1981) Enhancement of glutathione S-transferase activity of the mouse forestomach by inhibitors of benzo[a]pyrene induced neoplasia of the forestomach. *J. Natl Cancer Inst*., **66**, 769–771.
46. Kaderlik,K.R., Mulder,G.J., Turesky,R.J., Lang,N.P., Teitel,C.H., Chiarelli,M.P. and Kadlubar,F.F. (1994) Glucuronidation of N-hydroxy heterocyclic amines by human and rat liver microsomes. *Carcinogenesis*, **15**, 1695–1701.
47. Wortelober,H.M., de Kruif,C.A., van Iersel,A.A.J., Noordhock,J., Blaauboer,B.J., van Bladeren,P.J. and Falke,H.E. (1992) Effects of cooked Brussels sprouts on cytochrome P450 profile and phase II enzymes in liver and small intestinal mucosa of the rat. *Food Chem. Toxicol*., **30**, 17–27.
48. Pantuck,E.J., Pantuck,C.B., Anderson,K.E., Wattenberg,L.W., Conney,A.H. and Kappas,A. (1984) Effect of Brussels sprouts and cabbage on drug conjugation. *Clin. Pharmacol. Ther*., **2**, 161–169.
49. Knize,M.G., Salmon,C.P., Kulp,K.S. and Felton,J.S. (2001) Factors affecting heterocyclic aromatic amine intake and the metabolism of PhIP in humans. International Conference on Dietary Factors: Cancer Causes and Prevention, Vienna, Austria, Feb. 14–17.
50. Edenharder,R., Leopold,C. and Kies,M. (1995) Modifying actions of solvent extracts and vegetable residues on 2-amino-3,4-methylimidazo[4,5-*f*]quinoxaline induced mutagenesis in *Salmonella typhimurium. Mutat. Res*., **341**, 303–318.
51. Rauscher,R., Edenharder,R. and Platt,K.L. (1998) *In vitro* antimutagenic and *in vivo* anticlastogenic effects of carotenoids and solvent extracts from fruits and vegetables rich in carotenoids. *Mutat. Res*., **413**, 129–142.
52. Barcelo,S., Marce,K., Pfeifer,A.M.A. and Chipman,J.K. (1998) Production of DNA strand breaks by N-nitrosodimethylamine and 2-amino-3-methylimidazo[4,5-*f*]quinoline in THLE cells expressing human CYP isozymes and inhibition by sulforaphane. *Mutat. Res*., **402**, 111–120.
53. Verhoeven,D.T.H., Verhagen,H., Goldbohm,R.A., van den Brandt,P.A. and van Poppel,G. (1997) A review of mechanisms underlying anticarcinogenicity by *Brassica* vegetables. *Chem. Biol. Interact*., **103**, 79–129.
54. Chen,Y., Wang,W., Kong,A.N.T. and Tan,T. (1998) Molecular mechanisms of c-Jun N-terminal kinase-mediated apoptosis induced by anticarcinogenic isothiocyanates. *J. Biol. Chem.*, **273**, 1769–1775.
55. Yu,R., Mandlekar,S., Harvey,K.J., Ucker,D.S. and Kong,A.N.T. (1998) Chemopreventive isothiocyanates induce apoptosis and caspase-3-like protease activity. *Cancer Res*., **58**, 402–408.
56. Gamet-Payrastre,L., Li,P., Lumeau,S., Cassar,G., Dupont,M., Chevolleau,S., Gasc,N., Tulliez,J. and Terce,F. (2000) Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res*., **60**, 126–133.
57. Yang,Y., Conaway,C.C., Chiao,J.W., Wang,C., Amin,S., Whysner,J., Dai,W., Reinhardt,J. and Chung,F. (2002) Inhibition of benzoa[a]pyrene-induced lung tumori-genesis in A/J mice by dietary N-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res*., **62**, 2–7.
58. Nho,C.W. and Jeffery,E. (2001) The synergistic upregulation of phase II detoxification enzymes by glucosinolate breakdown products in cruciferous vegetables. *Toxicol. Appl. Pharmacol.*, **174**, 146–152.
59. de Vos,R.H. and Blijleven,W.G.H. (1988) The effect of processing conditions on glucosinolates in cruciferous vegetables. *Z. Lebensm. Unters. Forsch*., **187**, 525–529.
60. Howard,L.A., Jeffery,E.H., Wallig,M.A and Klein,B.P. (1997) Retention of phytochemicals in fresh and processed broccoli. *J. Food Sci*., **62**, 1098–1104.
61. Chen,C.W. and Ho,C.T. (1998) Thermal degradation of allyl isothiocyanate in aqueous solution. *J. Agric. Food. Chem.*, **46**, 220–223.
62. Maskell,I. and Smithard,R. (1994) Degradation of glucosinolates during *in vitro* incubations of rape seed meal with myrosinase (EC 3.2.3.1) and with pepsin (EC 3.4. 23. 1)-hydrochloric acid, and contents of porcine small intestine and caecum. *Br. J. Nutr*., **72**, 455–466.
63. Schwab,C.E, Huber,W.W., Parzefall,W,Hietsch,G., Kassie,F., Schulte-Hermann,R. and Knasmüller,S. (2000) Search for compounds that inhibit the genotoxic and carcinogenic effects of heterocyclic aromatic amines. *Crit. Rev. Toxicol*., **30**, 1–69.
64. Knasmüller,S. Steinkellner,H., Majer,B.J., Nobis,E.C., Scharf,G. and Kassie,F. (2002) Search for dietary antimutagens and anticarcinogens. Methodological aspects and extrapolation problems. *Food Chem. Toxicol.*, **40**, 1051–1062.

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