Metabolism
Clinical and Experimental

VOL 50, NO 10 OCTOBER 2001

Biochemical Effects of Dietary Intakes of Different Broccoli Samples. I. Differential Modulation of Cytochrome P-450 Activities in Rat Liver, Kidney, and Colon

Ole Vang, Henrik Frandsen, Kristian T. Hansen, Jørn N. Sørensen, Hilmer Sørensen, and Ole Andersen

Modulation of xenobiotic metabolism, including cytochrome P-450 (CYP) enzyme activities, due to dietary intakes of cruciferous vegetables, has been described in animals and humans, and the induction of CYP1A enzymes is suggested mainly to be related to the content of indolyl glucosinolates in these vegetables. The aim of the present study was to evaluate the effects on specific CYP activities of various broccoli samples containing different levels of glucosinolates. Groups of rats were fed 1 of 8 broccoli samples from 2 cultivars grown at different conditions. Thirteen different glucosinolates were quantified. The content of the 4 major glucosinolates, glucoraphanin (GRAP), glucoiberin, glucobrassicin (GB), and neoglucobrassicin (NeoGB) varied 5.6-, 2.7-, 3.2-, and 6.6-fold, respectively, among the broccoli samples. Dietary broccoli induced the CYP1A enzyme activities, 7-ethoxyresorufin-O-deethylase (EROD) and 7-methoxyresorufin-O-demethylase (MROD), in rat liver, weakly in colon, but not in kidney. In concordance, the hepatic metabolism of 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) to the proximate carcinogen N-OH-PhIP, a CYP1A-related activity, was enhanced by broccoli. The 7-pentoxyresorufin-O-depenthylase (PROD) activity, an assay for CYP2B1/2, was weakly induced in colon and kidney but not in liver by broccoli. The 2β-OH- and 6β-OH-testosterone hydroxylase activities were induced in liver microsomes, showing that broccoli increased CYP3A activity. The observed modulations of CYP activities depended clearly on the broccoli sample used, and significantly different responses were observed for different cultivars and growth conditions. These results indicate that modulation of CYP metabolism by broccoli may vary significantly in humans as well, as the content of glucosinolates and other active substances also varies between commercially available broccoli samples. The different effects depending on the vegetable sample eaten have to be considered in future experiments and dietary recommendations.

A HIGH DIETARY INTAKE of fresh fruit and vegetables decreases the risk of cancer in several organs, as has been shown both in epidemiological studies[1] and in animal experiments.[2] Based on these observations, a joint committee of the World Cancer Research Fund and the American Institute for Cancer Research, advised a daily intake of 400 to 800 g. of fruits and vegetables.[3]

The anticarcinogenic effects of fruit and vegetables as well as of substances isolated therefrom have been speculated to be primarily related to their modulating effect on the metabolism of exogenous and endogenous compounds. Various studies have shown induction as well as inhibition of different isoforms of cytochrome P-450 (CYP) and glutathione S-transferase by the proposed anticarcinogenic substances. The content of xenobiotic metabolizing enzymes is highest in liver, lung, and intestine. The same organs are those primarily exposed to xenobiotics.

Cruciferous vegetables have attracted special interest, as dietary intake of this single group of vegetables reduced the risk of cancer in 71% of available case-control studies.[4] Furthermore, these vegetables contain several substances that modulate biomarkers of cancer development when administered in

From the Department of Life Sciences and Chemistry, Roskilde University, Roskilde; National Food Agency of Denmark, Søborg; Department of Drug Metabolism, Novo Nordisk A/S, Måløv; Department of Fruit, Vegetable and Food Science, The Danish Institute of Agricultural Sciences, Årslev; and the Chemistry Department, Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Submitted September 29, 1999; accepted April 16, 2001.

Supported by grants from The Danish Food Ministry to O.V. (J.nr. 93S-2462-Å91-00014 and 93S-2462-Å91-00150), from The Commission of the European Communities, Agriculture and Fisheries to H.S. (FAIR CT 95-280), and from Norsk Hydro A/S and Wedell-Wedellsborg Foundation to O.V.

Address of reprint requests to Ole Vang, Department of Life Sciences and Chemistry, Roskilde University, PO Box 260, DK-4000 Roskilde, Denmark.

0026-0495/01/5010-0015$35.00/0
doi:10.1053/meta.2001.26743

pure form, ie, the glucosinolates and their breakdown products, indoles and isothiocyanates.[2] Few experimental animal studies on cancer chemoprevention have employed the intact glucosinolates or a mixture of compounds formed from the glucosinolates. A recent review concluded that indole-3-carbinol (I3C) may inhibit the risk of mammary, endometrial, forestomach, liver and tongue tumor formation.[5] Experiments with the pure indolyl and isothiocyanate compounds suggest that the glucosinolates are likely to be the most effective when present in a complex mixture.[6,7]

The purpose of the present study was to investigate possible relationships between the intake of a mixture of different glucosinolates in broccoli and the corresponding changes in CYP enzyme activities in rat liver, kidney, and colon. In an accompanying report, we examine the effects of the glucosinolates on these enzyme activities as well as different antioxidative defense enzymes[8,9] using principal component analysis to identify the potent modulators.

MATERIALS AND METHODS

Broccoli Samples

Eight different broccoli samples were used in the present study: 6 samples (*Brassica oleracea* L. var. *italica*) were grown during April through June in sandy loam and harvested in mid-June at the Department of Fruit, Vegetable and Food Science, The Danish Institute of Agricultural Sciences, Årslev, Denmark. Four of these samples were obtained by growing the cultivar *Shogun*, without use of pesticides, at different sulfur (S)- and nitrogen (N)-fertilizer levels (sample S0: 0 kg S and 170 kg N/hectare (ha); sample S1: 100 kg S and 170 kg N/ha; sample N0: 25 kg S and 20 kg N/ha; sample N1: 25 kg S and 400 kg N/ha). Two samples (E1 and H1) were cultivar *Emperor* and *Shogun*, respectively, grown without use of pesticides at 25 kg S and 170 kg N/ha. The remaining 2 samples (E2 and H2) were cultivar *Emperor* and *Shogun*, respectively, grown during June through August in silt loam, harvested in August, and obtained from an organic farmer (Nykøbing Sjælland, Denmark).

The plant materials were lyophilized to obtain dry powders for storage at room temperature. The broccoli powders were then mixed with phosphate-buffered saline (PBS; 80 mmol/L Na_2HPO_4, 20 mmol/L NaH_2PO_4, 100 mmol/L NaCl, pH 7.5; 0.25 g broccoli powder/mL buffer) and incubated at room temperature for 2 hours to degrade the glucosinolates.[10] After incubation, the broccoli samples were lyophilized once more. The contents of intact glucosinolates were determined before and after the degradation in duplicate by high-performance liquid chromatography (HPLC), as described previously,[11] for determination of the total amount of the specific glucosinolates and the degree of degradation. Subsequent experiments have shown that this hydrolysis in vitro seems not to be necessary.[6]

Animal Diet

The semisynthetic diet used in these experiments has been described previously.[8,12] The energy contribution was 18% from protein (casein), 70% from carbohydrate (icing sugar, dextrin, corn flour, and potato flour) and 12% from fat (olive oil, grape seed oil and coco oil). The P/S-ratio was 0.7, and 7% (wt/wt) cellulose was included as fiber. This basic diet was the control diet. To obtain the different broccoli diets, 10% (wt/wt) of the dried posthydrolysis broccoli samples was added to the basic diet by isocaloric exchange, calculated from the energy composition of the broccoli. The amount of added fiber was adjusted to assure that the energy composition and fiber contents of all diets were similar. The effects of the 8 different broccoli samples were tested in 2 independent experiments separated by 6 months. Broccoli samples S0, S1, N0, and N1 were tested in experiment 1 and samples E1, E2, H1, and H2 were tested in experiment 2.

Animals

In each experiment, 50 male Wistar rats (8 weeks old, 250 g body weight; Møllegaard, Skensved, Denmark) were kept as described previously.[8,12] The rats were fed the control diet for 3 weeks before random allocation to 5 groups, which received either the control diet or one of the 4 different broccoli diets for 1 additional week. The animals were killed by cervical dislocation and liver, kidneys, and colon were rapidly removed to ice. The following preparations were performed at

Table 1. Levels of Intact Glucosinolates in the Broccoli Samples

Glucosinolates	Broccoli Samples							
	S0	S1	N0	N1	E1	E2	H1	H2
	(mmol/g broccoli powder)							
Aliphatic glucosinolates								
Sinigrin	nd	nd	nd	0.22 ± 0.02	nd	nd	0.25 ± 0.18	nd
Gluconapin	0.43 ± 0.23	0.39 ± 0.01	0.34 ± 0.08	0.23 ± 0.06	nd	nd	0.44 ± 0.12	nd
Glucoiberverin	0.36 ± 0.08	0.44 ± 0.15	0.39	0.34 ± 0.08	0.10	nd	0.38 ± 0.11	nd
Glucoerucin	0.41 ± 0.19	0.66 ± 0.14	0.42 ± 0.04	0.40 ± 0.08	0.20	0.51 ± 0.13	0.43 ± 0.13	0.68 ± 0.18
Glucoiberin	7.83 ± 1.35	7.86 ± 0.05	7.24 ± 0.41	4.22 ± 0.17	nd	nd	7.3 ± 2.08	11.4 ± 2.43
Glucoraphanin	20.3 ± 2.22	20.3 ± 1.01	27.3 ± 2.86	13.0 ± 0.89	4.73 ± 0.068	9.02 ± 5.6	19.8 ± 5.92	26.4 ± 3.47
Glucocheirolin	0.31 ± 0.33	0.64 ± 0.80	0.099 ± 0.015	0.11 ± 0.015	nd	nd	0.081 ± 0.045	nd
Glucoerysolin	1.79 ± 1.07	2.27 ± 0.69	1.63 ± 0.24	0.81 ± 0.052	nd	nd	1.16 ± 0.33	nd
Aromatic glucosinolate								
Gluconasturtiin	0.59 ± 0.08	0.37 ± 0.13	0.42 ± 0.13	0.29 ± 0.073	0.46 ± 0.37	0.23 ± 0.20	0.52 ± 0.15	0.81 ± 0.22
Indolyl glucosinolates								
Glucobrassicin	4.40 ± 0.49	7.38 ± 0.43	3.27 ± 0.05	4.63 ± 0.59	4.35 ± 0.007	6.33 ± 0.72	4.01 ± 1.12	10.6 ± 5.04
Neoglucobrassicin	12.1 ± 7.17	16.1 ± 0.09	10.4 ± 0.79	9.06 ± 0.008	3.3 ± 0.77	5.05 ± 2.30	10.8 ± 3.0	21.7 ± 8.93
4-Hydroxyglucobrassicin	0.087 ± 0.05	0.061 ± 0.01	0.050 ± 0.006	0.027 ± 0.007	nd	nd	nd	nd
4-Methoxyglucobrassicin	0.83 ± 0.64	0.34 ± 0.35	0.45 ± 0.00	0.41 ± 0.013	0.37 ± 0.019	0.13 ± 0.036	0.49 ± 0.14	0.67 ± 0.38

NOTE. Glucosinolate levels were determined by HPLC as described previously,[11] prior to exposure to the endogenous myrosinase. Numbers are means of 2 determinations and the range.

Abbreviation: nd, nondetectable level.

0 to 4°C. The colonic mucosa was removed from the underlying tissue by scraping. Liver, kidneys, and colon mucosa were homogenized and the microsomes prepared.[12]

Enzyme Analysis

The 7-ethoxyresorufin-O-deethylase (EROD), 7-penthoxyresorufin-O-dephentylase (PROD), testosterone hydroxylase, and 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) metabolism were measured as described previously.[12] The 7-methoxyresorufin-O-demethylase (MROD) was measured by an endpoint method. The reaction mixture contained 0.5 mmol/L NADPH, 1.2 mg/mL albumin, 100 mmol/L Tris-HCl, pH 7.8, 200 mg microsomal proteins; 1 mL total volume for liver and 0.5 mL total volume for kidney and colon. After 2 minutes preincubation at 37°C, the reaction was initiated by addition of 1 μmol/L 7-methoxyresorufin, incubated at 37°C for 10 minutes, and stopped by the addition of 2.5 mL methanol. The proteins were sedimented by centrifugation and 10 μL 1*N* NaOH added to 1 mL of the supernatant and the fluorescence of the resorufin formed was measured at excitation 530 nm and emission 586 nm.

Statistics

Glucosinolate levels are given as mean and range, whereas enzyme activities are given as mean ± SEM. Numbers detected as outliers by the Systat version 6.0.1. computer program (SPSS, Chicago, IL) were not taken into account. Significant differences were tested using analysis of variance (ANOVA) following hypothesis testing using Scheffé's multiple contrasts test,[13] as there was no interaction at any level. In experiment 1, the contrasts were designed as follows: first—control diet versus broccoli diets (effect of broccoli), second—sample N0 versus sample N1 (effect of N fertilizer); and third—sample S0 versus sample S1 (effect of S fertilizer). In experiment 2, the contrasts were designed as follows: first—control diet versus broccoli diets (effect of broccoli), second—samples E1 and E2 together versus samples H1 and H2 together (effect of the cultivar), and third—samples E1 and H1 together versus samples E2 and H2 together (effect of growth condition).[13]

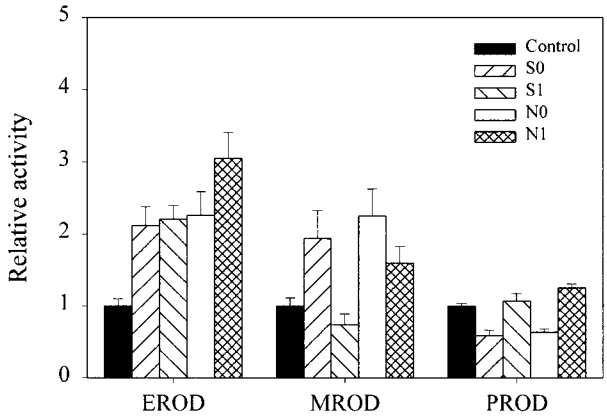
RESULTS

Contents of Glucosinolates

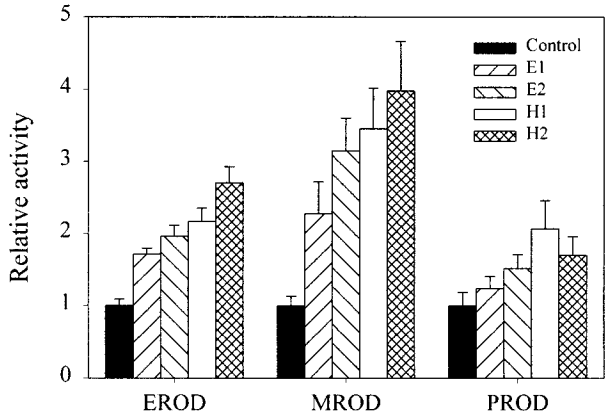
Eight different broccoli samples, differing in growth condition and in cultivars, were tested for their ability to affect CYP activities. The contents of specific glucosinolates in these samples were determined to allow study of possible relations between the glucosinolate content and modulation of CYP enzyme activities.

Among 13 glucosinolates identified in the broccoli samples using HPLC analysis, GRAP and glucoiberin were the major aliphatic glucosinolates in all *Shogun* cultivars, but not in *Emperor* samples (Table 1). The content of GRAP varied 5.8-fold between the different samples. The major indolyl glucosinolates were glucobrassicin (GB) and neoglucobrassicin (NeoGB), which varied 3.2- and 6.6-fold, respectively, in concentration among the 8 broccoli samples, but with much lower levels of NeoGB in *Emperor* compared with *Shogun*. More than 70% of the glucosinolates were degraded during the 2-hour autolysis of S0, S1, N0, N1, E1, and H1, whereas the glucosinolates were only degraded about 50% in sample E2 and H2 (data not shown).

A



B



C

	EROD	MROD	PROD
Experiment 1			
Control vs S0, S1, N0 and N1	P < 0.001	P = 0.046	NS
S0 vs S1	NS	P = 0.024	P < 0.001
N0 vs N1	NS	NS	P < 0.001
Experiment 2			
Control vs E1, E2, H1 and H2	P < 0.001	P < 0.001	NS
E1 and E2 vs H1 and H2	P < 0.001	P = 0.03	NS
E1 and H1 vs E2 and H2	P = 0.02	NS	NS

Fig 1. Relative CYP activities determined as EROD, MROD, and PROD in hepatic microsomes from rats after dietary exposure to different broccoli samples. Mean ± SEM for 8 to 10 animals in each group. (A) Experiment 1. Activities in control the group for EROD, MROD, and PROD were 22.2 ± 2.2, 14.8 ± 1.6, and 13.5 ± 0.5 pmol/min · mg protein, respectively. (B) Experiment 2. Activities in the control group for EROD, MROD, and PROD were 67.2 ± 4.3, 22.8 ± 3.0, and 10.8 ± 2.0 pmol/min · mg protein, respectively. (C) Overview of statistical differences in EROD, MROD, and PROD activities. NS, not significant.

Table 2. CYP Activities in Rat Liver Determined as Testosterone Hydroxylation Products After Dietary Intake of Broccoli: Mean ± SD of 9 to 10 Animals

	Activity (pmol metabolite formed/mg protein · min)					
	2α-OH-T	2β-OH-T	6β-OH-T	7α-OH-T	16α-OH-T	16β-OH-T
CYP enzyme	2C11	3A1/2	3A1/2	2A1	2B/2C	2B1/2
Experiment 1						
Control	1.3 ± 0.26	0.11 ± 0.027	1.5 ± 0.36	0.12 ± 0.01	1.5 ± 0.35	0.033 ± 0.0035
Sample S0	1.1 ± 0.40	0.16 ± 0.047	2.1 ± 0.65	0.14 ± 0.024	1.2 ± 0.35	0.040 ± 0.0096
Sample S1	1.0 ± 0.29	0.15 ± 0.025	1.9 ± 0.31	0.13 ± 0.019	1.2 ± 0.37	0.040 ± 0.0081
Sample N0	1.0 ± 0.26	0.17 ± 0.039	2.2 ± 0.50	0.16 ± 0.016	1.2 ± 0.36	0.041 ± 0.0078
Sample N1	1.0 ± 0.21	0.15 ± 0.015	1.9 ± 0.18	0.15 ± 0.021	1.2 ± 0.27	0.039 ± 0.0077
Experiment 2						
Control	1.11 ± 0.36	0.068 ± 0.016	1.1 ± 0.37	0.16 ± 0.026	1.2 ± 0.43	0.028 ± 0.0050
Sample E1	0.89 ± 0.16	0.12 ± 0.021	2.1 ± 0.32	0.17 ± 0.022	0.91 ± 0.26	0.035 ± 0.0051
Sample E2	0.88 ± 0.25	0.11 ± 0.027	1.8 ± 0.48	0.16 ± 0.021	0.87 ± 0.34	0.035 ± 0.0073
Sample H1	1.1 ± 0.25	0.11 ± 0.023	2.0 ± 0.48	0.18 ± 0.020	1.3 ± 0.36	0.038 ± 0.0085
Sample H2	0.83 ± 0.15	0.13 ± 0.030	2.2 ± 0.54	0.18 ± 0.023	0.97 ± 0.27	0.042 ± 0.0078

NOTE. The contrast design is described in the Materials and Methods.
Abbreviation: NS, not significant.

Effect of Dietary Broccoli on Weight and Organ Size

Changes in body, liver, or kidney weight were not observed among groups of rats fed the semisynthetic diet with 10% broccoli (wt/wt) for 1 week (data not shown).

Effects on Liver CYP Enzyme Activities

Hepatic EROD activity increased significantly up to 3-fold in livers from rats fed the broccoli diets for 1 week (Fig 1). MROD activity increased 2.3-fold after the intake of the broccoli diets. Furthermore, MROD activity increased differently depending on the broccoli sample used in the diet. Rats exposed to samples grown under low sulfur conditions (S0) had significantly higher MROD activity (2.5-fold) compared with S1-fed animals. Hepatic PROD activity, which is a marker for the CYP2B1/2 enzymes, was not significantly induced in rats fed broccoli diets. On the other hand, rats fed S0 and N0 broccoli did show almost 2-fold lower PROD activity compared with S1 and N1, respectively (Fig 1).

In experiment 2, hepatic EROD and MROD activities were increased 2.7- and 3.8-fold, respectively, by broccoli in the diet (Fig 1). An effect of growth conditions was observed: EROD activity was significantly higher in rats fed broccoli samples E2 and H2 compared with rats fed samples E1 and H1 (Fig 1). Likewise, the intake of *Shogun* cultivar increased EROD and MROD activities 1.2 to 1.5 times more than *Emperor* cultivar.

Activities of other hepatic CYP enzymes were determined using the testosterone hydroxylase assay (Tables 2 and 3). The formation of 2α-OH-T and 16α-OH-T, a CYP2C-related activity, was not affected by broccoli intake. On the other hand, the formation of 2β- and 6β-OH-T was increased significantly 1.5- to 1.8-fold by broccoli intake in both experiments, indicating increased CYP3A activity. The 7α-OH-T level, a CYP2A1 metabolite, and 16β-OH-T, a CYP2B metabolite, were increased in one experiment, but this increase was not reproducible (Tables 2 and 3).

Effect on Hepatic PhIP Metabolism

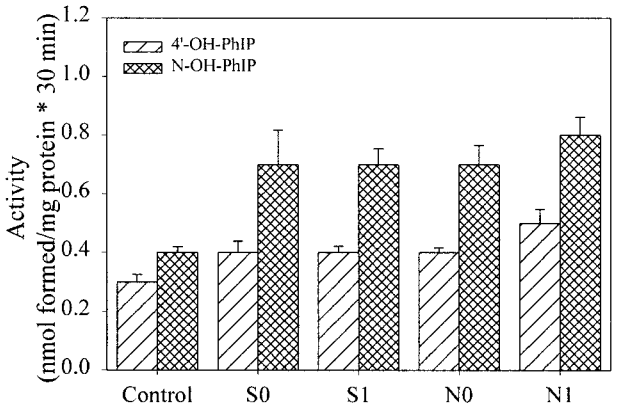
In vitro metabolism of the food mutagen PhIP by rat hepatic microsomes from experiment 1 (Fig 2) was analyzed to investigate whether dietary broccoli changes the formation of PhIP metabolites: 4'-OH-PhIP and N-OH-PhIP. The total levels of PhIP metabolites were increased by the broccoli diets. Formation of N'-OH-PhIP was increased 1.6- to 1.9-fold by broccoli, whereas a significantly different formation of 4'-OH-PhIP was

Table 3. Overview of Contrast Design Tests

	2α-OH-T	2β-OH-T	6β-OH-T	7α-OH-T	16α-OH-T	16β-OH-T
Experiment 1						
Control *v* S0, S1, N0, and N1	NS	$P < .001$	$P < .001$	$P < .01$	NS	NS
S0 *v* S1	NS	NS	NS	NS	NS	NS
N0 *v* N1	NS	NS	NS	NS	NS	NS
Experiment 2						
Control *v* E1, E2, H1, and H2	$P < .05$	$P < .001$	$P < .001$	NS	NS	$P < .001$
E1 and E2 *v* H1 and H2	NS	NS	NS	NS	NS	NS
E1 and H1 *v* E2 and H2	NS	NS	NS	NS	NS	$P < .05$

NOTE. The contrast design is described in the Materials and Methods.
Abbreviation: NS, not significant.

A



B

	4'-OH-PhIP	N-OH-PhIP
Control vs S0, S1, N0 and N1	NS	P < 0.01
S0 vs S1	NS	NS
N0 vs N1	P = 0.029	NS

Fig 2. Formation of PhIP metabolites in hepatic microsomes. (A) Mean ± SEM of 4′-OH-PhIP and N-OH-PhIP in 6 to 8 rats in each group from experiment 1. (B) Overview of statistical differences in the formation of 4′-OH-PhIP and N-OH-PhIP.

observed only in animals fed broccoli sample N1 compared with N0 (Fig 2).

Effect on Metabolizing Enzymes in Colon

The intestine is the second major organ of xenobiotic metabolism. The results in Fig 3 show that colonic EROD, MROD, and PROD activities were increased significantly in animals fed broccoli diets. EROD, MROD, and PROD were increased up to 4.1-, 6.4-, and 4.3-fold, respectively, in experiment 1. On the other hand, only small and not statistically significant increases were observed in experiment 2, where EROD, MROD, and PROD were increased 1.7-, 1.5-, and 2.3-fold, respectively (data not shown). In experiment 1, the 3 CYP activities were significantly different between animals fed broccoli samples S1 and S0 (Fig 3).

CYP Activity in Rat Kidney

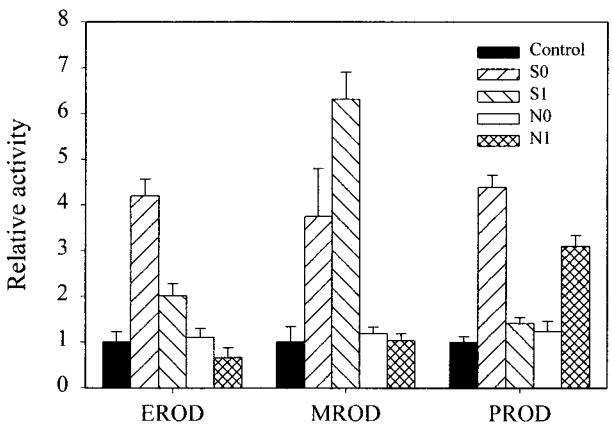
Only PROD was induced (4.5-fold) in rat kidney by broccoli diets in experiment 1 (Fig 4) simultaneously with a significant difference between animals fed broccoli samples N1 and N0. PROD was not induced in any of the groups of experiment 2 (data not shown).

DISCUSSION

Numerous plant-derived substances modulate xenobiotic metabolism. The effect of a compound could be quantitatively modified depending on whether it is present as a pure compound or part of a complex mixture.[7] Furthermore, the effects of various metabolites formed by degradation may differ from those of the parenteral substances. Therefore, experiments performed with pure compounds are of only limited value for evaluating their effects when present in a complex mixture. The present experiments were performed to study effects of dietary intakes of various broccoli samples on CYP enzyme activities. Differences in effect could, in part, be caused by different contents of glucosinolates. CYP expression is of major importance for the metabolism of chemical carcinogens, and the anticarcinogenic effects of various substances from fruit and vegetables have been suggested to be related to induction and inhibition of specific CYP enzymes.[14,15] In the present animal model, the increased CYP enzyme activities showed the combined effect of various compounds found in broccoli on the modulation of different levels of CYP enzyme regulation.

This study clearly shows that modulations of CYP activities depend on the broccoli sample administered, explaining why previous experiments have not consistently shown induction of, ie, hepatic CYP1A and 2B activities. The present study suggests that individual biochemical responses to broccoli intakes in humans are likely to vary significantly, as the commercial sources of broccoli also vary in the content of glucosinolates and other active substances. As the broccoli samples were not grown under completely identical conditions, this experiment is considered inadequate for analyzing the effect of growth conditions on the glucosinolate content.

A

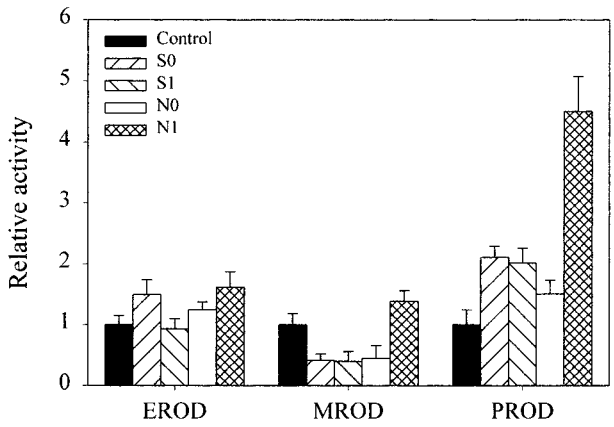


B

	EROD	MROD	PROD
Control vs S0, S1, N0 and N1	P < 0.001	P < 0.01	P < 0.001
S0 vs S1	P < 0.001	P < 0.01	P < 0.001
N0 vs N1	NS	NS	P < 0.001

Fig 3. Relative CYP activities determined as EROD, MROD, and PROD in rat colon. (A) Mean ± SEM for 7 to 10 animals in each group from experiment 1. EROD activity of group N1 and PROD activity of group N0 include only 4 and 5 rats, respectively. EROD, MROD, and PROD activities in the control group were 11.6 ± 2.6, 14.3 ± 4.8, and 14.6 ± 1.9 pmol/min · mg protein, respectively. (B) Overview of statistical differences in EROD, MROD, and PROD activities. None of the treatment groups in experiment 2 were statistically significantly different.

A



B

	EROD	MROD	PROD
Control vs S0, S1, N0 and N1	NS	NS	P < 0.01
S0 vs S1	NS	NS	NS
N0 vs N1	NS	P < 0.001	P < 0.001

Fig 4. Relative CYP activities determined as EROD, MROD, and PROD in rat kidney. (A) Mean ± SEM for 8 to 10 animals in each group from experiment 1. A reduced number of animals (5 or 6) are included in the MROD activity of group S1 and N1 and the PROD activity of control group and group N0. EROD, MROD, and PROD activities in the control group were 11.2 ± 1.7, 7.4 ± 1.3, and 3.5 ± 0.8 pmol/min · mg protein, respectively. (B) Overview of statistical differences in EROD, MROD, and PROD activities. None of the treatment groups in experiment 2 were statistically significantly different.

Before addition of the broccoli samples to the diet, the broccoli powder was incubated with a buffer to promote degradation of the glucosinolates, as the intact glucosinolates seem not to be active in vitro. On the other hand, our experiment performed after the present study with purified indolyl glucosinolates showed that intact glucosinolates are fully active in vivo without degradation prior to ingestion.[6] We therefore assume that a further degradation is taking place in the gut of the rats and that the differences in the rates of in vitro degradation are of minor significance. A more optimal condition is to mix the broccoli powder containing the intact glucosinolates directly into the animal diet without prior degradation, as several of the metabolites formed from the glucosinolates (isothiocyanates and indoles) are not stable, but will react with other components in the complex mixture. The degradation of the glucosinolates will then take place in the gut and the various metabolites will be formed in situ.

The enhanced hepatic EROD and MROD activities by the different broccoli samples indicate an up to 3.8-fold increase in CYP1A activity. In an earlier experiment, hepatic CYP1A mRNA, and CYP1A1 and CYP1A2 protein levels were previously found to be increased 2.4-, 7.5-, and 1.7-fold, respectively, in rats fed a similar amount of dietary broccoli[16] as in the present study.

The colonic CYP1A activity was also enhanced by the intake of broccoli, but the effect was not reproducible. Enhanced colonic CYP1A1 and unchanged CYP1A2 protein levels were previously observed in broccoli-fed rats[16] and the present increases in EROD and MROD activities observed in the present study are therefore likely to be caused by an increased CYP1A1 protein level. The lower response compared with that observed in liver may in part be explained as a simultaneous enzyme inhibition of the intestinal CYP1A as 3,3'diindolylemethane, I3C, N-methoxyindol-3-ylcarbinol, and other indoles inhibit EROD activity in vitro and PEITC inhibits CYP1A2-related metabolism.[17] However, experiments with another complex mixture, Savoy cabbage, have shown that EROD activity was enhanced several fold more in small intestine than in liver,[18] indicating the need for more focus on the actual components produced during degradation of glucosinolates.[10,19]

The food mutagen PhIP is metabolized primarily by CYP1A1 to 4'OH-PhIP and by 1A2 to N-OH-PhIP.[20] As dietary I3C reduced the PhIP-DNA adduct formation in rat colon[21,22] and dietary broccoli induced the rat hepatic and colonic CYP1A1 protein several fold over CYP1A2 protein, it has previously been proposed that broccoli may decrease the carcinogenic potency of PhIP by reducing the level of the ultimate carcinogenic metabolite, N-hydroxy-PhIP.[16] The present experiments show, nevertheless, that broccoli increases the formation of N-hydroxy-PhIP relative to the formation of 4'-hydroxy-PhIP, an effect previously found with I3C.[12] The observed chemopreventive effect of I3C on PhIP-DNA adduct formation in rat colon[21,22] may therefore be explained by other mechanisms than changing the CYP-mediated oxidation of PhIP.

Hepatic CYP2B1 and CYP2B2 activities were enhanced by some of the dietary broccoli samples in the present experiment, indicated by increased PROD activity and 16β-hydroxylation of testosterone. Several chemical compounds in cruciferous vegetables modulate CYP2B activities in rats when administrated in pure form. Wortelboer et al[23] found a strong induction of PROD activity following a few days exposure to I3C (30 mg/kg body weight) and PEITC inhibited rat hepatic PROD activity strongly, whereas sulforaphane, formed from GRAP, and allyl isothiocyanate, formed from sinigrin, only weakly inhibited the activities.[25]

Hepatic CYP3A activity, evaluated as 2β- and 6β-hydroxylation of testosterone, was significantly induced by broccoli. Previously, CYP3A protein levels were induced 3.8-fold by 120 mg I3C/ kg body weight in the diet for 7 days.[24]

The hepatic baseline EROD and MROD activities of the control animals differed up to 3-fold between experiment 1 and 2, but the relative effects of the dietary treatments were comparable. The difference may in part be explained by the fact that the 2 experiments were performed with a 6-month interval, and that different batches of diet components and animals were used.

In conclusion, the present experiments showed that 10% dietary broccoli induced CYP1A, 2B, and 3A activities in rat liver and CYP1A in colon. The experiments also clearly showed that the response depends on the broccoli sample used, indicating that intake of different commercial broccoli samples may affect the xenobiotic metabolizing capacity differently.

ACKNOWLEDGMENT

The authors wish to thank Dr H. Wallin, The National Institute of Occupational Health, Copenhagen, and Dr B. Søgaard, Royal Veterinary and Agricultural University, Frederiksberg, who took part in the initiation of the project. Dr J.B. Nielsen, Department of Community Health, Odense University, is thanked for assistance with the animal experiments. A. Maarup and J. Bayer are thanked for excellent technical assistance.

REFERENCES

1. Potter JD, Steinmetz K: Vegetables, fruit and phytoestrogens as preventive agents. IARC Sci Publ 139:61-90, 1996

2. Verhoeven DTH, Verhagen H, Goldbohm RA, et al: A review of mechanisms underlying anticarcinogenicity by brassica vegetables. Chem Biol Interact 103:79-129, 1997

3. WCRF/AICR: Food, Nutrition and Prevention of Cancer: A Global Perspective. Washington, DC, World Cancer Research Fund/ American Institute for Cancer Research, 1997

4. Steinmetz KA, Potter JD: Vegetables, fruit, and cancer. I. Epidemiology. Cancer Causes Control 2:325-357, 1991

5. Vang O, Dragsted L: Naturally Occurring Antitumourigens—III. Indoles. Copenhagen, Denmark, Nordic Council of Ministers, 1996

6. Bonnesen C, Stephensen PU, Andersen O, et al: Modulation of cytochrome P-450 and glutathione S-transferase isoform expression in vivo by intact and degraded indolyl glucosinolates. Nutr Cancer 33: 178-187, 1999

7. Staack R, Kingston S, Wallig MA, et al: A comparison of the individual and collective effects of four glucosinolate breakdown products from brussels sprouts on induction of detoxification enzymes. Toxicol Appl Pharmacol 149:17-23, 1998

8. Vang O, Rasmussen BF, Sørensen H, et al: The effect of dietary broccoli on oxidative stress biomarkers. Clin Chem 41:1910-1911, 1995

9. Vang O, Rasmussen BF, Andersen O: Combined effects of complex mixtures of potentially anti-carcinogenic compounds on antioxidant enzymes and carcinogen metabolizing enzymes in the rat. Cancer Lett 114:283-286, 1997

10. Agerbirk N, Bjergegaard C, Olsen CE, et al: Kinetic investigation of the transformations of indol-3-ylcarbinol into oligomeric indolyl compounds based on micellar electrokinetic capillary chromatography. J Chromatogr 745:239-248, 1996

11. Bjerg B, Sørensen H: Quantitative analysis of glucosinolates in oilseed rape based on HPLC of desulfoglucosinolates and HPLC of intact glucosinolates, in Wathelet J-P (ed): World Crops: Production, Utilization, Description. Glucosinolates in Rapeseeds: Analytical Aspects. Dodrecht, Netherlands, Martinus Nijhoff Kluwer Academic, 1987, pp 125-150

12. Vang O, Frandsen H, Hansen KT, et al: Modulation of drug-metabolising enzyme expression by condensation products of indole-3-ylcarbinol, an inducer in cruciferous vegetables. Pharmacol Toxicol 84:59-65, 1999

13. Zar JH: Biostatistical Analysis (ed 2). Englewood Cliffs, NJ, Prentice-Hall International, 1984

14. Manson MM, Hudson EA, Ball HW, et al: Chemoprevention of aflatoxin B1-induced carcinogenesis by indole-3-carbinol in rat liver—Predicting the outcome using early biomarkers. Carcinogenesis 19: 1829-1836, 1998

15. Stresser DM, Bjeldanes LF, Bailey GS, et al: The anticarcinogen 3,3'-diindolylmethane is an inhibitor of cytochrome P-450. J Biochem Toxicol 10:191-201, 1995

16. Vang O, Jensen H, Autrup H: Induction of cytochrome P-450IA1, IA2, IIB1, IIB2 and IIE1 by broccoli in rat liver and colon. Chem Biol Interact 78:85-96, 1991

17. Smith TJ, Guo ZY, Guengerich FP, et al: Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by human cytochrome P450 1A2 and its inhibition by phenethyl isothiocyanate. Carcinogenesis 17:809-813, 1996

18. Kwon CS, Grose KR, Riby J, et al: In vivo production and enzyme-inducing activity of indolo[3,2-b]carbazole. J Agric Food Chem 42:2536-2540, 1994

19. Agerbirk N, Olsen CE, Sørensen H: Initial and final products, nitriles, and ascorbigens produced in myrosinase-catalyzed hydrolysis of indole glucosinolates. J Agric Food Chem 46:1563-1571, 1998

20. Wallin H, Mikalsen A, Guengerich FP, et al: Differential rates of metabolic activation and detoxication of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by different cytochrome P450 enzymes. Carcinogenesis 11:489-492, 1990

21. Guo D, Schut HAJ, Davis CD, et al: Protection by chlorophyllin and indole-3-carbinol against 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-induced DNA adducts and colonic abberant crypts in the F344 rat. Carcinogenesis 16:2931-2937, 1995

22. Wargovich MJ, Chen C-D, Jimenez A, et al: Aberrant crypts as a biomarker for colon cancer: Evaluation of potential chemopreventive agents in the rat. Cancer Epidemiol Biomarkers Prev 5:355-360, 1996

23. Wortelboer HM, van der Linden EC, De Kruif CA, et al: Effects of indole-3-carbinol on biotransformation enzymes in the rat: In vivo changes in liver and small intestinal mucosa in comparison with primary hepatocyte cultures. Food Chem Toxicol 30:589-599, 1992

24. Stresser DM, Bailey GS, Williams DE: Indole-3-carbinol and β-naphthoflavone induction of aflatoxin B-1 metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin B-1 in the rat. Drug Metab Dispos 22:383-391, 1994