Biochemical Effects of Dietary Intake of Different Broccoli Samples. II. Multivariate Analysis of Contributions of Specific Glucosinolates in Modulating Cytochrome P-450 and Antioxidant Defense Enzyme Activities

Ole Vang, John Mortensen, and Ole Andersen

Dietary broccoli exposure modulates various cytochrome P-450 (CYP)-associated activities and antioxidant defense enzyme activities in liver, colon, and kidney of rats. We present an analysis by the partial least-square method (PLS) of the contribution of single glucosinolates in modulating xenobiotic metabolizing and antioxidant defense enzyme activities. Generally, modulation of colonic enzyme activities was well described (58% to 75%) by models consisting of 3 principal components (PCs). The indolyl glucosinolates were not the only major contributors to the regulation of colonic 7-ethoxyresorufin O-deethylase (EROD) and 7-methoxyresorufin O-demethylase (MROD) activities, as would be expected from results of previous experiments testing the pure compounds, glucobrassicin (GB), neoglucobrassicin (NeoGB), and 4-methoxyglucobrassicin (4-MeOGB). In hepatic and renal microsomes, the modulation of enzyme activities could be partly described for hepatic and renal 7-pentoxyresorufin O-deethylase (PROD) activities (42% to 44%, 3 to 4 PCs), hepatic superoxide dismutase activity (45%, 2 PCs), and renal glutathione peroxidase (GSH Px) and glutathione reductase (GSSG Red) activities (43%, 3 PCs). These results indicate that substances other than glucosinolates in the complex mixtures modulate hepatic EROD, MROD, GSH Px, and GSSG Red activities or that the active glucosinolate metabolites vary in their systemic disposition.

DIETS HIGH IN FRUITS and vegetables reduce the incidence of cancers in epidemiological studies and animal models.[1] Fruits and vegetables contain numerous chemical components, of which several have anticarcinogenic activities when administered in pure form at high concentrations in animal models.[2,3] The anticarcinogenic effect of fresh fruit and vegetables is, however, unlikely to be caused by single or few substances, but is more likely caused by the combined effect of a multitude of substances.[4] The combined effect of the various active compounds in a mixture, eg, broccoli, may be quite different from the effect of the single compounds. Additive, antagonistic, and synergistic interactions between the different substances may occur. Furthermore, the carcinogenic process contains several distinct steps, and the different anticarcinogenic compounds act simultaneously on several mechanisms.[5]

Cruciferous vegetables are a dominant source for human intakes of glucosinolates, which have been shown to modulate biomarkers of cancer development when administered in pure form.[3] Most experimental animal studies on cancer chemoprevention in relation to these compounds, including modulation of xenobiotic metabolism, employed the pure indolyl and isothiocyanate products formed during degradation of the glucosinolates rather than the intact glucosinolates in complex mixtures. Several studies showed induction of various cytochrome P-450 (CYP) enzymes by some of these substances, whereas others report the major effect to be an inhibition of other CYP enzymes. The content of CYP enzymes is highest in liver, lung, and intestine, which are also the organs primarily exposed to and responsible for the metabolism of xenobiotics.

Multivariate data analysis, in particular partial least-square (PLS) regression, including principal component (PC) analysis, is a powerful method for analysis of complex data structures, originally developed for analysis of complex chromatographic data.[6] During the last decade, PC analysis has been employed to examine complex data in all areas of the natural sciences, including human and ecological toxicology,[7,8] disease heritability analyses,[9] DNA sequence analysis,[10] taxonomy,[11] and pathology and pathophysiology.[12,13] To our knowledge, the present report is the first employing PC analysis of biochemical effects of complex dietary components. PC analysis is a linear projection method that helps visualize all the information contained in a data table followed by a regression analysis;[14] using PLS we attempted to identify possible relationships between activities of CYP enzymes and the antioxidative defense enzymes superoxide dismutase, glutathione peroxidase (GSH Px), and glutathione reductase (GSSG Red), and dietary intake of a complex mixture of different glucosinolates from broccoli.[5,15,16] This was done by quantifying the modulating effects of different dietary broccoli samples and identifying those substances that most efficiently affected enzyme activity levels.

MATERIALS AND METHODS

The design of experiments and the specific analyses are described in detail in an accompanying report.[15] In brief, 8 different broccoli samples were used in the present studies (composed of 2 independent experiments): 4 of the samples were obtained by growing the cultivar *Shogun* at different sulfur (S)- and nitrogen (N)-fertilizer levels. In the second experiment, 2 separate cultivars, *Emperor* and *Shogun*, respectively, were grown under different conditions ("organic" and "conventional") and at 2 different locations. The levels of 13 glucosinolates in the 8 different broccoli samples were determined by high-performance liquid chromatography (HPLC), as described previously.[17]

The effects of broccoli diets were tested in 2 separate experiments, each containing 1 control group and 4 treatment groups (10% [wt/wt] broccoli in diet), with 10 rats in each group. Various enzymatic analyses were performed in liver, colon, and kidney. In all three organs, 7-ethoxyresorufin O-deethylase (EROD), 7-methoxyresorufin O-de-

From the Department of Life Sciences and Chemistry, Roskilde University, Roskilde, Denmark.

Submitted September 21, 1999; accepted April 16, 2001.

Supported by grants from The Danish Food Ministry (Jnr. 93S-2462-Å91-00014 and 93S-2462-Å91-00150).

Address reprint requests to Ole Vang, Department of Life Sciences and Chemistry, Roskilde University, Universitetsvej 1, PO Box 260, DK-4000 Roskilde, Denmark.

0026-0495/01/5010-0016$35.00/0

doi:10.1053/meta.2001.26744

Table 1. Glucosinolate Contents in the Eight Broccoli Samples

	Broccoli Samples							
Glucosinolates	S0	S1	N0	N1	E1	E2	H1	H2
	(mmol/g broccoli powder)							
Sinigrin	nd	nd	nd	0.22 ± 0.02	nd	nd	0.25 ± 0.18	nd
GNAP	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
GRAP	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
GNAS	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
GB	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
NeoGB	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-OHGB	0.087 ± 0.05	0.061 ± 0.01	0.050 ± 0.006	0.027 ± 0.007	nd	nd	nd	nd
4-MeOGB	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. Data from Vang et al.[15]

Abbreviation: nd, nondetectable level.

methylase (MROD), and 7-pentoxyresorufin O-dependentylase (PROD) activities were measured.[15] Together with these phase I metabolic activities, various antioxidant defense enzyme activities were reported earlier.[16]

Statistics

The PLS analysis presented here was based on the levels of intact glucosinolates in the broccoli samples, as our recent data showed that ingestion of both degraded and intact indolyl glucosinolates by rats induces hepatic CYP1A and CYP2B.[18] The enzyme activities were listed as relative values to those in the control groups to make the activities of the 2 separate experiments comparable.

All glucosinolate level data were normalized using mean normalization to get the data in the same scaling,

$$X(i,k) = \frac{X(i,k)}{|\bar{x}(i,\cdot)|}$$

where X(i,k) denotes the value of sample i, variable k, and X(i, ·) denotes the ith sample vector.

PLS was performed using The Unscrambler software, version 6.11 from CAMO ASA (Oslo, Norway). The PLS method models both the X- and Y-matrices, glucosinolates and enzyme activities, respectively, simultaneously to find the latent variables in X that will best predict the latent variables in Y. A cross-validation method was used on centered data set, weighted by 1/SD. The PLS method assumes data linearity. Although this is plausible for dose-response relationship with a single compound, it is not strictly guaranteed for the dose-response relationship for a complex mixture.

The aim of the analysis was to make models where the number of contributing factors, 13 glucosinolates, were reduced to few PCs. To avoid noise in modeling, only factors that extracted more than 5% of the sample variance were taken into account.

RESULTS

Eight broccoli samples, which differed in growing conditions and in cultivars, were tested for their ability to alter different CYP activities[15] and antioxidative defense enzymes[5,16] in rat liver, colon, and kidney. The levels of specific glucosinolates (Table 1 and Fig 1) were determined in these samples to study relations between glucosinolate content and modulation of the enzyme activities using a PLS method. The optimum is the instance where a few components describe as much as possible of the data variance.

Models for Regulation of CYP Enzyme Activities

Of the 3 organs analyzed, the activities of the colon were well described using models including 3 PCs, where 65% and 75% of the variances of colonic EROD and MROD activities, respectively, were described (Table 2). On the other hand, no models could be established for the hepatic and renal EROD and MROD activities. The PROD activities of all 3 organs were partly explained by models consisting of 3 or 4 components describing 44% to 58% of the data variation, which, surprisingly, in liver and kidney were much better than the description of EROD and MROD activities.

A colonic EROD activity model was established having 3 PCs describing 65% of the data variance with PC-1 and PC-2 describing most of the model (58%) (Table 3). Gluconapin (GNAP), glucocheirolin, and 4-hydroxyglucobrassicin (4-OHGB) are the major contributors in the positive direction of PC-1, whereas gluconasturtiin (GNAS), glucoerysolin and neoglucobrassicin (NeoGB) are the major contributors of PC-2.

In addition, colonic MROD activity was described by a 3-PC model, explaining 75% of the variation, and the indolyl glucosinolates contributed to the different PCs in the MROD model: 4-OHGB to PC-1, NeoGB to PC-2, and 4-methoxyglucobrassicin (4-MeOGB) to PC-3. Glucocheirolin plays a major role in the regulation of MROD activity as it is the main contributor to all 3 PCs. Besides glucocheirolin, other aliphatic glucosinolates contributed to the model for MROD: glucoerysolin and sinigrin are the major contributors to PC-1, whereas glucoraphanin (GRAP) was a major factor for PC-2 (Table 4). This model predicted well the combined effect of the 13 glucosinolates on colonic MROD activity with a high correlation

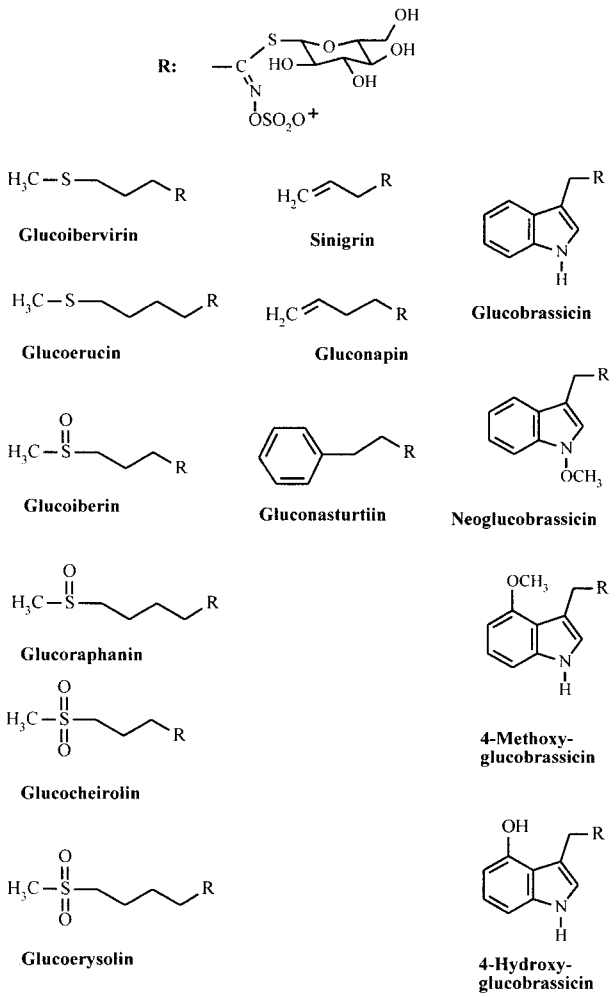

Fig 1. Structures of the glucosinolates identified in the broccoli samples and used for PLS modulation.

coefficient comparing the observed and predicted values, $r = .91$ (Fig 2).

The major contributors to colonic PROD activity were 4-OHGB, 4-MeOGB, glucoerysolin, and GNAP to PC-1, and glucoerysolin, GNAS, GRAP, and NeoGB to PC-2 (data not shown).

Models for Regulation of Antioxidant Defense Enzyme Activities

About 67% of the colonic GSH Px and GSSG Red activities were described by models consisting of 3 components (Table 2). Colonic GSH Px activity was found to be largely regulated by glucoerysolin and 4-MeOGB in PC-1, GRAP, glucoerucin, and glucobrassicin (GB) in PC-2, and glucoibervirin and 4-MeOGB in PC-3 (Table 5). In the case of colonic GSSG-Red activity, glucoerucin is a major contributor to both PC1 and PC2. Besides glucoerucin, glucoibervirin, glucocheirolin, and 4-OHGB were the major contributors to PC-1 in this model, GRAP, GB, and NeoGB to PC-2, and sinigrin and glucoibervirin to PC-3 (Table 6).

The variation of renal and hepatic GSH Px and GSSG Red activities were less well described by the content of glucosinolates analyzed here (Table 2).

DISCUSSION

Our present knowledge about toxicological and biochemical effects of xenobiotics is based mainly on studies on effects of the pure compounds. This may be relevant to elucidate the mechanism of action for a compound, but evaluation and extrapolation of these results to the effects of the same compound in a complex mixture require great caution.

Several natural dietary substances belonging to different chemical classes are known to modulate the metabolism of xenobiotics in animal models testing pure substances at relatively high concentrations. Therefore, the present analysis was performed to extract knowledge about modulatory effects of single compounds present in a complex mixture as broccoli. Traditional statistical analysis is not suited for analysis of multifactorial interactions. Accordingly, we employed PLS analysis in the present study.

Several unexpected observations were made performing PLS analysis of the effects of the thirteen glucosinolates on different enzyme activities in liver, colon, and kidney of rats. According to the literature, indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC), degradation products of GB and GNAS, respectively, enhance the hepatic CYP1A protein level and most likely the EROD and MROD activities,[19] whereas several aliphatic glucosinolates are known to inhibit various CYP activities. In vitro and cell culture studies have shown that

Table 2. Overview of Model Generation for the Analyzed Hepatic, Renal, and Colonic Biomakers Using PLS Analysis

	No. of PC*	Explained Variance†
Liver		
EROD	0	0%
MROD	2	30%
PROD	3	42%
Superoxide dismutase	2	45%
GSH Px	2	22%
GSSG Red	1	12%
Colon		
EROD	3	65%
MROD	3	75%
PROD	3	58%
GSH Px	3	67%
GSSG Red	3	68%
Kidney		
EROD	0	0%
MROD	1	7.4%
PROD	4	44%
GSH Px	3	42%
GSSG Red	3	42%

NOTE. The models were based on the levels of 13 glucosinolates in the 8 broccoli samples, and the relative enzyme activities measured in exposed rats.

*Number of PCs integrated in the model, ie, components that add >5% to the description of the variance.

†How much of the total data variance could be explained by this model.

Table 3. Explained Variance and Loading Weights Describing a Model for Modulation of Rat Colonic EROD Activity by 13 Glucosinolates in Eight Broccoli Samples

	Principal Components*		
Explained Variance†	PC-1 28%	PC-2 30%	PC-3 6.8%
	X-Loading Weights‡		
Sinigrin	−0.24	−0.11	0.26
GNAP	**0.38**	−0.08	**0.37**
Glucoibervirin	0.16	−0.31	−0.28
Glucoerucin	−0.10	0.24	**−0.52**
Glucoiberin	0.31	−0.15	0.32
GRAP	0.10	−0.34	−0.02
Glucocheirolin	**0.37**	0.01	−0.14
Glucoerysolin	−0.15	**−0.51**	−0.33
GNAS	−0.08	**0.40**	0.15
GB	−0.23	0.23	−0.13
NeoGB	−0.06	**0.36**	0.11
4-MeOGB	0.23	0.18	**−0.36**
4-OHGB	**0.62**	0.22	−0.17

*The model consists of linear PCs, which in combination explain the contributions of the 13 glucosinolates on the modulation of colonic EROD activity.

†The explained variance for each PC >5% is shown.

‡Loadings describe the data structure in terms of variable correlations. Each glucosinolate has a loading on each PC. It reflects both how much the variable contributed to this PC, and how well the PC takes into account the variable's variation over the data points. Loadings can range between −1 and 1, and large loading indicates high contribution of the variable to the PC. Bold numbers indicate a significant contribution to the specific component, ie, >10% of the loading sum.

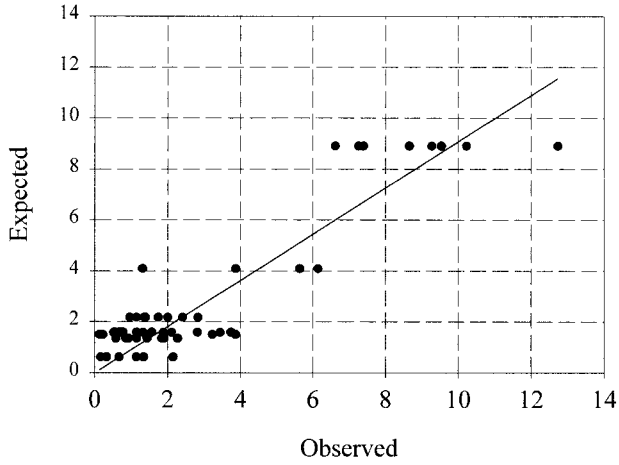

Fig 2. Correlation of predicted *v* measured data of colonic MROD activity. Using the model generated for colonic MROD activity, the MROD activity was calculated for each sample based on the content of 13 glucosinolates in the 8 broccoli samples. The correlation coefficient was *r* = .91 and the root mean square error was 1.22.

indoles may inhibit CYP1A1-related EROD activity. It was therefore suggested that the observed hepatic EROD and MROD activities in rats fed broccoli-containing diets were regulated mainly by indolyl glucosinolates and GNAS.[15] However, no clear model could be generated using PLS for hepatic or renal EROD and MROD activities on the basis of the intact or degraded glucosinolate levels, and neither the GB, NeoGB, nor GNAS were found to regulate these activities substantially (Table 2). This unexpected observation may be explained in 2 ways: first, broccoli contains other compounds than the 13 glucosinolates analyzed here that may contribute more to the regulation of hepatic and renal CYP1A activities. S-methylcysteinsulfoxide is one candidate that is found in broccoli at levels comparable with the total glucosinolate level.[20] The contents of S-methylcysteinsulfoxide in these samples are, however, not known. The effects of S-methylcysteinsulfoxide on EROD or MROD activities are not known, but it is spontaneously con-

Table 4. Explained Variance and Loading Weights Describing a Model for Modulation of Rat Colonic MROD Activity by 13 Glucosinolates in Eight Broccoli Samples

	Principal Components*		
Explained Variance†	PC-1 32%	PC-2 31%	PC-3 12%
	X-Loading Weights‡		
Sinigrin	**−0.32**	−0.16	0.24
GNAP	0.16	−0.33	−0.19
Glucoibervirin	0.10	−0.35	−0.15
Glucoerucin	−0.01	0.28	**−0.59**
Glucoiberin	0.20	−0.27	−0.03
GRAP	0.06	**−0.36**	0.20
Glucocheirolin	**0.70**	**0.41**	**0.38**
Glucoerysolin	**0.34**	−0.10	−0.02
GNAS	−0.21	0.29	0.15
GB	−0.15	0.27	−0.28
NeoGB	−0.08	**0.36**	0.08
4-MeOGB	−0.13	−0.01	**0.41**
4-OHGB	**0.36**	−0.02	−0.27

NOTE. See Table 3 for further details.

Table 5. Explained Variance and Loading Weights Describing a Model for Modulation of Rat Colonic GSH Peroxidase Activity by 13 Glucosinolates in Eight Broccoli Samples

	Principal Components*		
Explained Variance†	PC-1 24%	PC-2 18%	PC-3 25%
	X-Loading Weights‡		
Sinigrin	0.31	0.23	0.20
GNAP	0.11	−0.35	−0.11
Glucoibervirin	0.29	−0.09	**0.56**
Glucoerucin	0.26	**0.43**	0.13
Glucoiberin	0.15	−0.35	−0.15
GRAP	−0.18	**−0.38**	0.19
Glucocheirolin	0.29	−0.18	−0.20
Glucoerysolin	**0.59**	−0.07	0.10
GNAS	−0.32	0.21	−0.22
GB	0.01	**0.36**	−0.02
NeoGB	0.09	0.30	−0.26
4-MeOGB	**−0.38**	0.05	**0.63**
4-OHGB	−0.05	−0.27	−0.01

NOTE. See Table 3 for further details.

Table 6. Explained Variance and Loading Weights Describing a Model for Modulation of Rat Colonic GSSG Reductase Activity by 13 Glucosinolates in Eight Broccoli Samples

	Principal Components*		
Explained Variance†	PC-1 27%	PC-2 24%	PC-3 17%
	X-Loading Weights‡		
Singirin	−0.15	0.11	**0.70**
GNAP	0.22	−0.25	0.08
Glucoibervirin	**0.38**	−0.07	**0.51**
Glucoerucin	**0.38**	**0.52**	0.02
Glucoiberin	0.16	−0.29	0.05
GRAP	−0.17	**−0.47**	−0.04
Glucocheirolin	**0.50**	−0.04	−0.23
Glucoerysolin	0.29	−0.15	0.13
GNAS	−0.29	0.20	−0.20
GB	0.07	**0.41**	−0.01
NeoGB	−0.02	**0.33**	−0.02
4-MeOGB	−0.05	0.04	0.25
4-OHGB	**0.41**	−0.08	−0.23

NOTE. See Table 3 for further details.

verted to dimethyldisulfide and other disulfides[21] and several disulfide compounds have been shown to increase hepatic EROD and MROD activities in rats.[22] Moreover, flavonoids inhibit the CYP1A activity in vitro,[23] and we suggest that other potent modulators of hepatic CYP1A activities could well be identified in broccoli in future studies. Second, the glucosinolates may be digested and transported differently which may affect the results. It should be noted that PLS assumes linearity in dose-response and a nonlinear response of the measured enzyme activities to exposure to indolyl glucosinolates is possible.

In contrast to the hepatic and renal enzyme activities, colonic EROD, MROD, GSH Px, and GSSG Red activities were well described by 3-component models. The high degree of explanation of the variance in the models for colonic enzyme activities as compared with the effects in liver and kidney is most likely caused by the fact that colon mucosa is exposed directly to the components found in a broccoli diet.

The models for colonic EROD (Table 3) and MROD (Table 4) include substantial contributions from gluccheirolin, NeOGB, 4-MeOGB, and 4-OHGB, but several other glucosinolates contribute significantly. The effects of indolyl glucosinolates were less than expected, considering the inducing effect of pure GB, NeoGB, and 4-MeOGB on hepatic EROD[18] and the induction of colonic CYP1A1 mRNA by I3C.[24] The present results indicate that also 4-OHGB contributes significantly to the regulation of colonic MROD activity in spite of the low level of 4-OHGB in broccoli.[15] No effect of glucocheirolin on CYP metabolism has been reported, but recently glucocheirolin was shown to inhibit the growth of human cells.[25] The present results indicate that further experiments might focus on the modulatory effects of 4-OHGB and glucocheirolin and the combinatory effects of the glucosinolates on CYP enzyme activities.

Broccoli was demonstrated to induce colonic GSH Px and GSSG Red activities, depending on the broccoli samples used[5,16] and the present analysis allowed establishment of three PC-models for the colonic GSH Px (Table 5) and GSSG Red activity (Table 6) in contrast to hepatic and renal activities. In general, the present data indicate that aliphatic glucosinolates were the major modulators of colonic GSSG Red and GSH Px. At present, only PEITC and a sulforaphane-like substance are known to induce colonic GSH Px,[26-28] whereas data on the levels of colonic GSSG Red activity following exposure to dietary substances are lacking. Hepatic GSSG Red activity is enhanced by I3C and iberin, and more by a combination of I3C, crambene, iberin, and PEITC, but not by PEITC alone.[25]

The PROD activity of liver and kidney appeared to be more closely regulated by the glucosinolates investigated than the activities of EROD and MROD (Table 2). One reason could be that aliphatic isothiocyanates inhibited PROD more effectively than EROD. The IC_{50}s for PEITC were 47, 46, and 1.8 mmol/L for EROD, MROD, and PROD, respectively, and alkyl isothiocyanates, sulforaphane, and allyl isothiocyanate were very weak inhibitors.[29] In addition, hepatic CYP2B levels are induced by treatment with I3C and PEITC, but not by sinigrin.[19]

In conclusion, PLS analysis allowed breakdown of the effects of the glucosinolates on different colonic enzyme activities. No clear model could be created for the same enzyme activities in liver and kidney. As these enzyme activities may have a modulatory effect on chemical carcinogenesis, the present observation agrees with the fact that the risk of colon cancer, but not that of kidney or liver cancer, has been demonstrated to depend on the composition of the diet in the western countries. Furthermore, no single glucosinolate may account for the modulation of colonic CYP and antioxidant defense enzyme activities.

REFERENCES

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

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

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

4. Helzlsouer KJ, Block G, Blumberg J, et al: Summary of the Round Table Discussion on Strategies for Cancer Prevention—Diet, Food, Additives, Supplements, and Drugs. Cancer Res 54:S2044-S2051, 1994

5. 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

6. Norli HR, Esbensen K, Westad F, et al: Chemometric evaluation of urinary steroid profiles in doping control. J Steroid Biochem Mol Biol 54:83-88, 1995

7. Calleja MC, Persoone G, Geladi P: Human acute toxicity prediction of the first 50 MEIC chemicals by a battery of ecotoxicological tests and physicochemical properties. Food Chem Toxicol 32:173-187, 1994

8. Kaiser KLE, Esterby SR: Regression and cluster analysis of the

acute toxicity of 267 chemicals to six species of biota and the octanol/water partition coefficient. Sci Total Environ 109-110:499-514, 1991

9. Ott J, Rabinowitz D: A principal-components approach based on heritability for combining phenotype information. Hum Hered 49:106-111, 1999

10. Sub SO, Jones KG, Blackwell M: A Group I intron in the nuclear small subunit rRNA gene of *Cryptendoxyla hypophloia*, an ascomycetous fungus: Evidence for a new major class of group I introns. J Mol Evol 48:493-500, 1999

11. Ortiz S, Bujan M, Rodriguez-Oubina J: A revision of European taxa of Hyacinthoides section Somera (Hyacinthaceae) on the basis of multivariate analysis. Plant Syst Evol 217:163-175, 1999

12. Armstrong RA, Cairns NJ, Lantos PL: Quantification of pathological lesions in the frontal and temporal lobe of ten patients diagnosed with Pick's disease. Acta Neuropathol 97:456-462, 1999

13. Wright IC, Sharma T, Ellison ZR, et al: Supra-regional brain systems and the neuropathology of schizophrenia. Cereb Cortex 9:366-378, 1999

14. Wold S, Esbensen K, Geladi P: Principal component analysis. Chem Intell Lab System 2:37-52, 1987

15. Vang O, Frandsen H, Hansen KT, et al: Biochemical effects of dietary intake of different broccoli samples. I. Modulation of cytochrome P-450 activities in rat liver, kidney and colon. Metabolism 50:1123-1129, 2001

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

17. 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

18. 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

19. Manson MM, Ball HW, Barrett MC, et al: Mechanism of action of dietary chemoprotective agents in rat liver: Induction of phase I and II drug metabolizing enzymes and aflatoxin B1 metabolism. Carcinogenesis 18:1729-1738, 1997

20. Hansen M, Laustsen AM, Olsen CE, et al: Chemical and sensory quality of broccoli (*Brassica oleracea* L. var *Italica*). J Food Qual 20:441-459, 1997

21. Stoewsand GS: Bioactive organosulfur phytochemicals in Brassica oleracea vegetables—A review. Food Chem Toxicol 33:537-543, 1995

22. Haber D, Siess MH, Dewaziers I, et al: Modification of hepatic drug-metabolizing enzymes in rat fed naturally occurring allyl sulphides. Xenobiotica 24:169-182, 1994

23. Siess MH, Leclerc J, Canivenc-Lavier MC, et al: Heterogenous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. Toxicol Appl Pharmacol 130:73-78, 1995

24. Vang O, Jensen MB, Autrup H: Induction of cytochrome P450IA1 in rat colon and liver by indole-3-carbinol and 5,6-benzoflavone. Carcinogenesis 11:1259-1263, 1990

25. Nastruzzi C, Cortesi R, Esposito E, et al: In vitro antiproliferative activity of isothiocyanates and nitriles generated by myrosinase-mediated hydrolysis of glucosinolates from seeds of cruciferous vegetables. J Agric Food Chem 48:3572-3575, 2000

26. van Lieshout EM, Posner GH, Woodard BT, et al: Effects of the sulforaphane analog compound 30, indole-3-carbinol, D-limonene or relafen on glutathione S-transferases and glutathione peroxidase of the rat digestive tract. Biochim Biophys Acta 1379:325-336, 1998

27. van Lieshout EMM, Ekkel MPC, Bedaf MMG, et al: Effects of dietary anticarcinogens on rat gastrointestinal glutathione peroxidase activity. Oncol Rep 5:959-963, 1998

28. 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

29. Conaway CC, Jiao D, Chung F-L, et al: Inhibition of rat liver cytochrome P450 isozymes by isothiocyanates and their conjugates: A structure-activity relationship study. Carcinogenesis 17:2423-2427, 1996