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Safety, Tolerance, and Metabolism of Broccoli Sprout Glucosinolates and Isothiocyanates: A Clinical Phase I Study

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Abstract: *Broccoli sprouts are widely consumed in many parts of the world. There have been no reported concerns with respect to their tolerance and safety in humans. A formal phase I study of safety, tolerance, and pharmacokinetics appeared justified because these sprouts are being used as vehicles for the delivery of the glucosinolate glucoraphanin and its cognate isothiocyanate sulforaphane [1-isothiocyanato-(4*R*)-(methylsulfinyl)butane] in clinical trials. Such trials have been designed to evaluate protective efficacy against development of neoplastic and other diseases. A placebo-controlled, double-blind, randomized clinical study of sprout extracts containing either glucosinolates (principally glucoraphanin, the precursor of sulforaphane) or isothiocyanates (principally sulforaphane) was conducted on healthy volunteers who were in-patients on our clinical research unit. The subjects were studied in three cohorts, each comprising three treated individuals and one placebo recipient. Following a 5-day acclimatization period on a crucifer-free diet, the broccoli sprout extracts were administered orally at 8-h intervals for 7 days (21 doses), and the subjects were monitored during this period and for 3 days after the last treatment. Doses were 25 μmol of glucosinolate (cohort A), 100 μmol of glucosinolate (cohort B), or 25 μmol of isothiocyanate (cohort C). The mean cumulative excretion of dithiocarbamates as a fraction of dose was very similar in cohorts A and B (17.8 ± 8.6% and 19.6 ± 11.7% of dose, respectively) and very much higher and more consistent in cohort C (70.6 ± 2.0% of dose). Thirty-two types of hematology or chemistry tests were done before, during, and after the treatment period. Indicators of liver (transaminases) and thyroid (thyroid-stimulating hormone, T3, and T4) function were examined in detail. No significant or consistent subjective or objective abnormal events (toxicities) associated with any of the sprout extract ingestions were observed.*

Introduction

Broccoli and other cruciferous vegetables are widely consumed and are believed to play an especially important role in contributing to the well-recognized health benefits of plant-based diets in reducing the risk of cancer and many common chronic degenerative diseases (1,2). The Cruciferae (Brassicaceae) plant family is characterized by a unique phytochemistry: their high content (often several percent by weight) of glucosinolates, which are β-thioglucoside *N*-hydroxysulfates with more than 120 unique side chains derived from common amino acids (3). There is growing evidence that the protective effects of crucifers against disease may be attributable in large measure to their content of glucosinolates. Glucosinolates in plant cells are accompanied by, but physically segregated from, a β-thioglucosidase (myrosinase), which, when released by crushing of plant cells such as by chewing or food preparation, hydrolyzes the glucosinolates, principally to isothiocyanates (mustard oils) (3) (Fig. 1). This hydrolysis is also mediated by the microflora of the mammalian gastrointestinal tract (4). The principal glucosinolate contained in broccoli is glucoraphanin, which is hydrolyzed by myrosinase to sulforaphane [1-isothiocyanato-(4*R*)-(methylsulfinyl)butane] (5–7).

Substantial cellular protection against electrophile, oxidative, and inflammatory stresses in animals and their cells is afforded by the induction of a variety of cytoprotective (so-called phase 2) genes. This strategy holds much promise in reducing the risk of cancer and various chronic degenerative diseases (8,9). Guided by bioassays for phase 2 inducer activity, sulforaphane was isolated from broccoli in 1992 (5). It is the principal and extremely potent inducer of the phase 2 response and blocks the formation of DMBA-evoked mammary tumors in rats (10) as well as other tumors in various

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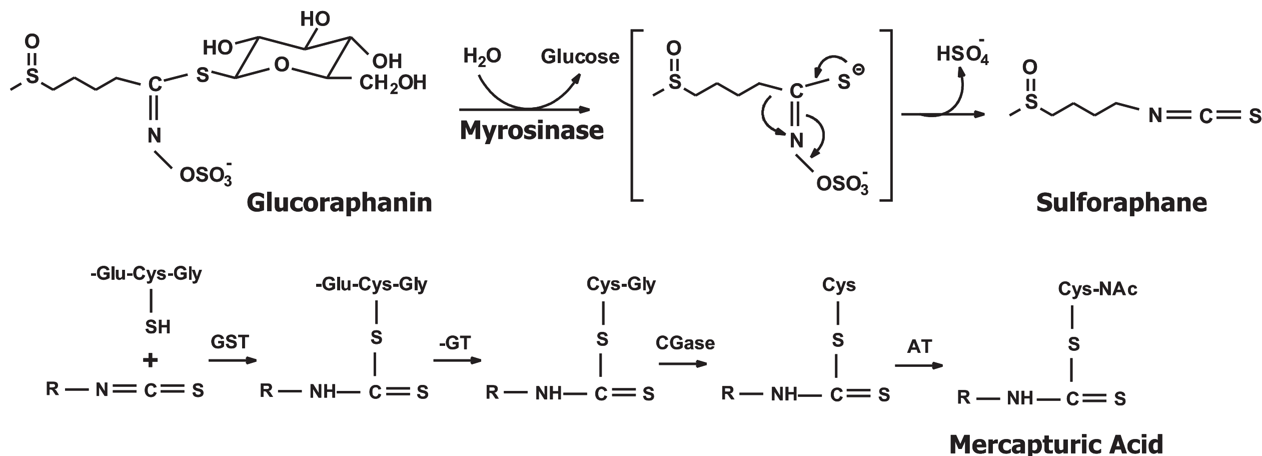

Figure 1. Myrosinase catalyzes the hydrolysis of glucoraphanin to sulforaphane. Sulforaphane is metabolized by conjugation with glutathione (catalyzed by glutathione *S*-transferases) and the sequential catalytic activities of γ-glutamyltransferase (γ-GT), cysteinylglycinase (CGase), and *N*-acetyltransferase (AT) ultimately to form mercapturic acid. Sulforaphane and all of the glutathione-derived conjugates (dithiocarbamates) are detected by the cyclocondensation reaction with 1,2-benzenedithiol.

animal models. The hydrolytic conversion of glucosinolates to isothiocyanates is important because the isothiocyanates and not their glucosinolate precursors are the active moieties that react with Keap1, the sensor for inducers that signals transcription of phase 2 genes (9,11,12).

Isothiocyanates are metabolized in animal tissues by the mercapturic acid pathway, which involves the initial conjugation with glutathione catalyzed by glutathione transferases followed by successive cleavage of the γ-glutamyl residue (by γ-glutamyltransferase), removal of the glycine residue (by cysteinylglycinase), and finally *N*-acetylation (by *N*-acetyltransferases) to give *N*-acetylcysteine conjugates (mercapturic acids) (Fig. 1). Sulforaphane and the aforementioned conjugates, which are collectively known as dithiocarbamates (DTCs), can all be quantified by cyclocondensation with the vicinal dithiol 1,2-benzenedithiol to give rise to 1,3-benzodithiole-2-thione, which has highly favorable properties for spectroscopic determinations (13,14). The cyclocondensation reaction was used to standardize the sulforaphane and glucoraphanin content of the doses and to quantify the urinary DTC excretion (4,7,15).

Because of the widely varying content of glucoraphanin in mature broccoli, 3-day-old sprouts were grown from selected seeds to contain precisely defined levels of this phytochemical (6). These sprouts are a convenient vehicle for human delivery of standardized amounts of glucoraphanin and sulforaphane. Broccoli sprouts are widely available and have been consumed by large numbers of individuals in the United States, Japan, and other parts of Asia. A phase I evaluation appeared justified because of the prospects of very-long-term consumption by healthy individuals to achieve health protection. Studies to assess chemoprotection in humans have already been reported from both our and other laboratories (16,17).

The present phase I study examines the safety and tolerance of repeated oral administration of extracts of broccoli sprouts, containing either glucosinolates or sulforaphane, in three cohorts of healthy volunteers who were hospitalized on our clinical research unit under stringently controlled conditions. After a "washout" period on a crucifer-free diet, the subjects (three experimental and one control per cohort) received specified doses for 7 days (every 8 h) of the aforementioned phytochemicals and underwent repeated clinical evaluation as well as a comprehensive battery of tests. These studies were designed to establish tolerance and safety, as well as to determine pharmacokinetics, in anticipation that broccoli sprouts will be used in human trials to establish efficacy against a wide variety of conditions involving oxidative stress and electrophile toxicity. Such trials are likely to involve administration to large numbers of individuals for prolonged periods of time. The study reported here is a limited phase I study involving a small number of individuals for a relatively short period of time. It can now be followed by larger and more prolonged studies. We emphasize these limitations.

Methods and Materials

Study Design

This study examined the safety and tolerance of repeated oral administration of aqueous extracts of broccoli sprouts containing either glucosinolates or isothiocyanates (produced from glucosinolates by deliberate myrosinase hydrolysis) on three cohorts of healthy volunteers who were hospitalized in our research unit for 17–19 days and fed a controlled diet that was devoid of crucifers and other sources of inducers of phase 2 enzymes. The low levels of inducers were confirmed by direct analysis of representative samples of the diet and by finding negligible excretion of DTC metabolites derived from glucosinolates and isothiocyanates in the urine.

The study was a randomized, placebo-controlled, double-blind trial on 12 healthy volunteers. Three successively studied cohorts consisted of four subjects each, of which three received an active extract and one received a matching placebo extract. After a 5-day acclimatization period, the volunteers received orally at 8-h intervals for 7 days (21 doses total) broccoli sprout extracts containing either glucosinolate or isothiocyanate preparations in the following doses: cohort A, 25 μmol of glucosinolates (subjects 1–4); cohort B, 100 μmol of glucosinolates (subjects 5–8); and cohort C, 25 μmol of isothiocyanates (subjects 9–12). The range of doses of glucosinolates and isothiocyanates was selected to be comparable with reasonable consumptions of broccoli sprouts. Thus, for broccoli sprouts that normally contain 6 μmol of GS per gram fresh weight, the administered daily doses of 75 and 300 μmol of GS would correspond to 12.6 and 50.4 g of sprouts, respectively.

The protocols for these studies were approved by our institutional review board. Individuals were screened by physical examination and by blood and urine analyses. The screening tests excluded individuals who were smokers or were pregnant or who tested positive for HIV or hepatitis A, B, or C. Routine blood and urine tests were performed, and the results had to fall within normal limits for eligibility. Blood tests included Hct, Hgb, RBC, WBC (including differential), platelets, and reticulocytes. Blood chemistries included prothrombin time, partial thromboplastin time, Na^+, K^+, Cl^-, CO_2, glucose, creatinine, blood urea nitrogen, albumin, direct/total bilirubin, alkaline phosphatase, transaminases (alanine aminotransferase/aspartate aminotransferase, ALT/AST), γ-glutamyltransferase, T3, T4, and thyroid-stimulating hormone (TSH). Urines were subjected to both microscopic and chemical analyses, including measurement of DTC and creatinine.

Thirty-two quantitative parameters (see Table 1) were determined on 6 occasions during the study in each of the 12 subjects for a total of 2,304 planned determinations. All but five samples were successfully collected and analyzed. Bloods were collected at 7 am on two occasions before treatment (Days 0 and 6), twice during treatment (Days 9 and 13), and twice after treatment (Days 16 and 19). Subjects were discharged on Day 17 or 19.

Hospitalization Schedule

Subjects were admitted on Day 0 and hospitalized until Day 17 (cohorts A and B) and returned on Day 19 for final blood tests. For cohort C, the hospitalization period was extended until after bloods were obtained on Day 19. Days 0–5 were a stabilization and "washout" period on a crucifer-free diet. Doses of broccoli sprouts were administered orally under supervision at 8-h intervals (7 am, 3 pm, and 11 pm) from 7 am on Day 6 to 11 pm on Day 12 (7 days, 21 doses). The placebo closely matched the active doses in appearance. Compliance was complete, as confirmed by the urinary analyses of DTCs and creatinines. Urines were collected in 8-h aliquots from 7 am on Day 1 to 11 pm on Day 16 inclusively (48 samples per subject). All urines were collected and analyzed. The analyst for DTCs was not involved in the clinical evaluation of the subjects.

Identity of Subjects and Dose Assignments

- Cohort A: subjects 1–4 (subject 1 received placebo).
- Cohort B: subjects 5–8 (subject 7 received placebo).
- Cohort C: subjects 9–12 (subject 10 received placebo).

All subjects were male except for subject 7. The ages and ethnicities of the 12 subjects are recorded in Table 2.

Preparation of Diet

Considerations involved in the design, preparation, and testing of the diet are fully described by Shapiro et al. (7). Briefly, all components of the diets were tested in advance to verify their lack of phase 2 enzyme-inducing properties. Typical diets consumed by individuals in a single day contained the equivalent of the inducer activity of no more than 2 μmol of sulforaphane. It is notable that the minimum daily dose of sulforaphane equivalent (administered in cohort A) was 75 μmol. The diet consisted of 6 separate days of unique meals that were cycled to allow feeding for the full 17–19 days of the in-patient phase of the study. The diet was bland by design and contained no cruciferous vegetables, onions, leeks, garlic, chives, caffeine-containing beverages, or condiments (for example, horseradish, mustard, wasabi, soy sauce, or ketchup). It presented an extreme overabundance of calories (5,000 kcal/day), designed to prevent volunteers from going to extraordinary lengths to secure forbidden foods. Protein, carbohydrate, and fat represented 17%, 43%, and 40% of calories supplied to subjects. Neither uneaten food nor actual caloric intake was monitored.

Broccoli Sprout Preparations

Growth of broccoli sprouts: Sprouts were grown in an isolated room dedicated to growing and processing sprouts. This room contained a filtered source of deionized water, running hot and cold water, a floor drain, a cart for growing sprouts equipped with lights and a timer-controlled water-misting spray device, an autoclavable 4-l stainless steel blender, a balance, and a large (100 l) stainless steel, steam-jacketed commercial food preparation kettle.

Seeds were stored in vermin-proof cabinets. All equipment destined for contact with sprouts was either autoclaved, washed with hot water and soap, or disinfected with dilute Clorox bleach or ethanol solutions. Operators were instructed on appropriate washroom and hand-sanitizing procedures. Vinyl gloves were used for most operations. No laboratory chemicals were stored or used in the food preparation room. After the initial steam kettle extraction steps, subsequent dose preparation was executed in the General Clinical Research Center research kitchen. Personnel practices in-

Table 1. All Abnormal Hematology and Chemistry Values[a]

			Abnormal Values					
			Pretreatment		Intratreatment		Posttreatment	
Blood Test	Range of Normal	Subject (gender)	Day 0	Day 6	Day 9	Day 13	Day 16	Day 19
Red cell count (million/mm^3)	m 4.40–5.90;	9 (m)				4.39		
	f 3.80–5.20							
Hemoglobin (g/dl)	m 14.0–18.0;	5 (m)	13.5					13.3
	f 11.5–16.0	6 (m)						13.8
		7 (f) PL					11.4	10.6
		8 (m)	13.3					
		9 (m)	13.1	13.7	13.2	12.5	13.3	13.4
Hematocrit (%)	m 40.0–52.0;	5 (m)						39.7
	f 35.0–47.0	7 (f) PL						32.3
		8 (m)	39.5					
		9 (m)				37.5		
Polymorphonuclear leukocytes (%)	42–78	1 (m) PL	83					
		9 (m)				37		
		12 (m)					39	
Lymphocytes (%)	15–45	1 (m) PL	11					
		7 (f) PL			47			
		9 (m)				53		
Monocytes (%)	0–12	12 (m)						14
Eosinophils (%)	0–7	2 (m)						9
		4 (m)				15		12
		10 (m) PL		13	10		11	
		12 (m)					16	
Reticulocytes (%)	0.5–2.4	1 (m) PL				2.5		
		4 (m)		2.5				
		6 (m)	2.5	2.5				
		7 (f) PL						2.5
		9 (m)						2.5
		11 (m)				2.8		
Prothrombin time (s)	8.8–11.8	4 (m)		8.5				
		7 (f) PL			8.1			
Partial thromboplastin time (s)	23.2–32.0	11 (m)		33.6	33.2		32.8	
Aspartate aminotransferase (IU/l)	m 8–45;	5 (m)						49
	f 8–35	8 (m)						107
Alanine aminotransferase (IU/l)	m 0–45;	6 (m)				65	59	62
	f 0–40	12 (m)				48		
Glucose (mg/dl)	65–115	1 (m) PL						64
		3 (m)	156					
		4 (m)	118					
		8 (m)	199		120	118		133
Bicarbonate (mEq/l)	18–30	10 (m) PL				32		
Total triiodothyronine (ng/dl)	60–181	9 (m)	182					
Free thyroxin (ng/dl)	0.8–1.5	2 (m)						0.78
		5 (m)						0.78
		7 (f) PL					0.73	
Thyroid-stimulating hormone (μIU/ml)	m 0.40–4.20;	1 (m) PL			5.2		5.10	
					0			
	f 0.70–6.40	9 (m)						5.20
		11 (m)			5.3	4.40	4.40	7.60
					0			

a: Abbreviations are as follows: m, male; f, female; PL, placebo recipient; IU, International Unit. Normal hematology and chemistry test values: no abnormal values were found at any time point for the following determinations: white cell count (3,900–11,000 per mm^3); bands (0–10%); basophils (0–2%); atypical lymphocytes (0–4%); platelets (140,000–440,000 per mm^3); γ-glutamyltransferase (m 11–65, f 5–65 IU/ml); alkaline phosphatase (30–130 IU/ml); total bilirubin (0.2–1.5 mg/dl); direct bilirubin (0.0–0.5 mg/dl); albumin (3.7–5.2 g/dl); blood urea nitrogen (m 8–25, f 5–25 mg/dl); creatinine (m 0.5–1.6, f 0.5–1.4 mg/dl); sodium (134–146 mEq/l); potassium (3.5–5.5 mEq/l); chloride (95–110 mEq/l).

Table 2. Urinary Excretion of Dithiocarbamates in Subjects Dosed With Broccoli Sprout Glucosinolates and Isothiocyanates[a]

							Mean 8-h DTC Excretion[b]		Cumulative Excretion DTC	
Cohort	Subject	Age	Gender	Race	Individual Dose (µmol/8 h)	Total Dose (µmol)	% of Dose (±SD)	Range (%)	(µmol)	(% of dose)
A	1	40	m	B	Placebo					
	2	46	m	B	25	525	15.24 ± 3.71	(7.72–22.40)	85.4	16.3
	3	57	m	W	25	525	10.09 ± 2.34	(5.87–15.68)	53	10.1
	4	45	m	W	25	525	27.15 ± 8.09	(16.03–41.16)	142.5	27.1
B	5	45	m	B	100	2100	30.06 ± 5.99	(16.90–38.30)	653.3	31.1
	6	48	m	W	100	2100	19.16 ± 6.49	(10.0–32.50)	421.4	20.1
	7	21	f	B	Placebo					
	8	45	m	B	100	2100	7.22 ± 2.27	(4.14–13.40)	161.7	7.70
C	9	46	m	B	25	525	67.86 ± 21.30	(13.8–81.30)	368	70.1

a: Abbreviations are as follows: DTC, dithiocarbamate; m, male; B, Afro-American; W, Caucasian; f, female.
b: Mean quantities of DTC excreted during 8-h intervals (expressed as % of 8-h dose). These values represent the 21 collections obtained during the dietary intervention (Days 5–11). The range column shows the highest and lowest values for each subject.

cluded the following: no eating, drinking, or smoking in the facility; personal hygiene and appropriate food-handling procedures were strictly enforced. Broccoli sprout production:

Seeds of broccoli *(Brassica oleracea* var. *italica*) cultivar "DeCicco", which were certified not to have been exposed to pesticides or other chemicals used in seed treatment, were surface disinfected for 15 min with a 25% aqueous solution of Clorox bleach (20,000 ppm sodium hypochlorite) containing a trace of Alconox detergent. The seeds were stirred every 5 min to ensure that the sanitizing solution was thoroughly mixed with the seeds. Hypochlorite was removed by exhaustive rinsing with distilled water. The seeds were then spread in monolayers on inclined, perforated plastic trays on a commercial-type sprout cart. Seeds were misted with filtered potable water for 30 s about six times per hour and were illuminated continuously by overhead cool-white fluorescent lights. The room temperature was approximately 20°C.

Dose preparation: Growth was arrested after 3 days by plunging sprouts directly into boiling water in a steam-jacketed kettle, returning to a boil, and stirring for at least 5 min. This time is sufficient to inactivate the endogenous myrosinase present in the sprouts. The "glucosinolate" preparation consists of the supernatant, drawn off by passing the contents of the kettle through a commercial colander-type strainer and then through a fine nylon mesh and cooled. To obtain the "isothiocyanate" preparation, a homogenate of 7- to 10-day-old daikon sprouts (as a potent source of myrosinase), prepared according to Shikita et al. (18), was added to this glucosinolate-rich extract at a level of 2% of the weight of the broccoli sprouts initially used to make the boiled aqueous extract. This preparation with added daikon sprouts was homogenized and maintained at 37°C for 2 h. It was then filtered through a reusable stainless-steel coffee filter and fine nylon mesh. Placebo preparations were made from the fifth boiling water extract of broccoli sprouts, which were processed as described for the active preparations. A single batch of sprout extracts was used for each cohort. Repeated analyses disclosed no loss of active material during several weeks. Aliquots of all four preparations (active glucosinolates and isothiocyanates and placebo glucosinolates and isothiocyanates) were retained for analyses (including microbiological testing) and then placed in food-grade plastic jugs and frozen in a lockable, dedicated freezer until analyses were complete. The methods for isothiocyanate and glucosinolate analyses have been described (4,7,15). Following analyses, homogenates were thawed rapidly; weighed into plastic-capped, food storage cups; and frozen in a lockable, dedicated freezer until required for consumption by subjects. All preparations were tested in a commercial bacteriology laboratory (Strasburger and Siegel, Hanover, MD) for *Escherichia coli* 0157:H7, *Salmonella* sp., and *Listeria monocytogenes* and found to be negative.

Analyses of Sprout Preparations

The analytical procedures for qualitative and quantitative assay of isothiocyanates and glucosinolates and for the in vitro phase 2 inducer activity measurements of these preparations have been described (7). The isothiocyanate/glucosinolate characteristics of each preparation are shown in Table 3. Briefly, glucosinolate profiles were initially assessed by paired-ion high-performance liquid chromatography (19) and confirmed by hydrophilic interaction chromatography (20), and total glucosinolates were measured before and after their conversion to isothiocyanates with purified myrosinase (18) by the cyclocondensation reaction (13–15). Total isothiocyanates as well as residual glucosinolates and isothiocyanates were calculated from differences between samples before and after myrosinase treatment. The induction of NAD(P)H:quinone acceptor oxidoreductase 1 activity in cultured cells was measured by

Table 3. Analytical Composition of Administered Preparations

Placebo Preparation	
Residual isothiocyanate	≤0.01 µmol/ml
Residual glucosinolate	≤0.03 µmol/ml
Dose administered	7.5 ml (corresponding to 25-µmol dose) 30 ml (corresponding to 100-µmol dose)
Isothiocyanate Preparation	
Total isothiocyanate	3.3 µmol/ml
Residual glucosinolate	0.1 µmol/ml
Dose administered	7.5 ml
Glucosinolate Preparation[a]	
Total isothiocyanate	0.02 µmol/ml
Total glucosinolate	3.3 µmol/ml
Glucosinolate profile	
Glucoraphanin	73.5%
Glucoerucin	21.7%
4-Hydroxyglucobrassicin	1.9%
Glucobrassicin	1.8%
Neoglucobrassicin	1.1%

a: It is notable that these glucosinolate preparations, unlike many other broccoli sprout glucosinolate extracts, contained no glucoiberin (3).

the "Prochaska bioassay" and was compared with the potency of pure sulforaphane (6,21,22).

Results and Discussion

Dithiocarbamate Excretion

All subjects completed the study. DTC levels for all subjects were measured by the cyclocondensation reaction (4,7,15) on every 8-h urine collection obtained during the period of hospitalization. The results are plotted as total micromoles of DTCs per 8-h collection (Fig. 2). The values for the 21 8-h urine collections obtained from each subject during the intervention period are shown in Table 2, which gives mean (±SD) and ranges for each subject. All urine samples obtained from placebo recipients contained less than <0.1 µmol of DTC per 8-h collection, demonstrating complete compliance with the prescribed diet. The mean 8-h DTC excretion rate (expressed as percentage fraction of each

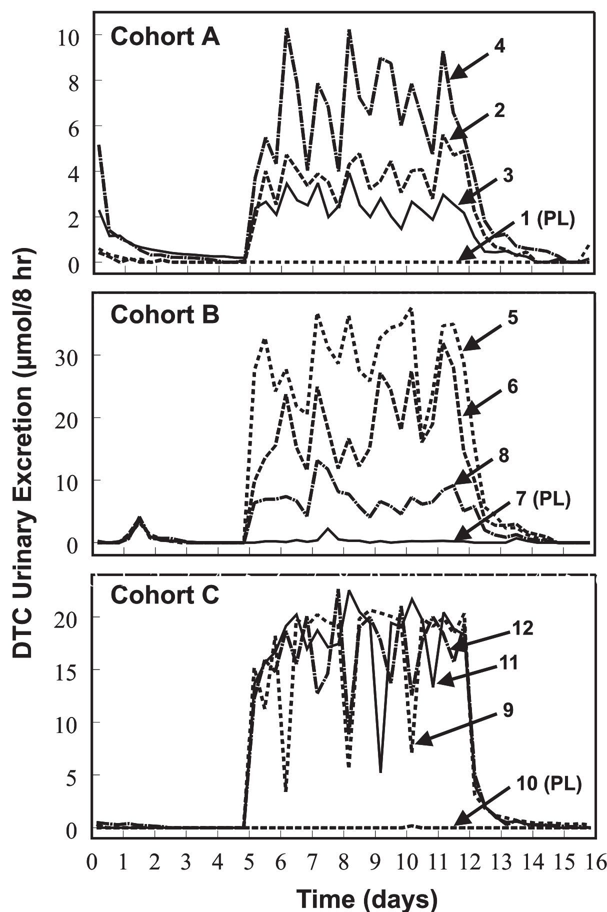

Figure 2. Urinary excretion of dithiocarbamates (DTCs, expressed as micromoles per 8-h collection) for all 12 subjects (cohort A, upper; cohort B, middle; cohort C, lower panel) from Day 0 (admission to hospital) to Day 16. Cohort A received 25 µmol and cohort B received 100 µmol of glucosinolates, whereas cohort C received 25 µmol of isothiocyanates, every 8 h, from 7 am on Day 5 to 11 pm on Day 11. The almost-flat lines in each panel are the DTC excretions by placebo recipients. The numbers associated with each line identify the subjects (see text). Note different scales of *y*-axes. In cohort B, the small DTC excretion peaks observed on Days 1/2 in all subjects and on Days 7/8 and 13/14 in the placebo recipient were attributable to the presence on those days of a different hamburger lot in the diet that tested positive for inducers. The values are shown for each 8-h period, that is, 21 determinations during the 7-day period of intervention (Days 5–11).

8-h dose) as well as the cumulative excretion during the entire 7-day period were calculated. The three subjects who received 25-µmol doses of the glucosinolate preparation (525 µmol total dose over 7 days) excreted 10.1%, 16.3%, and 27.1% of the total dose. The three subjects who received 100 µmol of glucosinolate preparation (2,100 µmol total dose) excreted 7.7%, 20.1%, and 31.1% of the total dose. The mean cumulative excretions expressed as fraction of dose for all six subjects was 18.7 ± 9.4% (±SD). There was a large (almost fourfold) difference in total excretion among individuals. This variation probably reflects 1) individual differences in conversion of glucosinolates to isothiocyanates, for example, quantitative or qualitative differences in the gastrointestinal microflora; 2) polymorphisms in glutathione *S*-transferases that affect DTC formation from isothiocyanates (23); and 3) other factors that contribute to bioavailability, biotransformation, and excretion. Determinants of the efficiency of the microflora in converting glucosinolates to isothiocyanates could be complex and be affected by diet, host genetic factors, ethnicity, gender, gastrointestinal transit time, and even the enterohepatic circulation. Such differences in total excretion among individuals are in agreement with our recent observations on 100 healthy human subjects who received broccoli sprout extracts that contained the equivalent of 400 µmol of glucoraphanin daily for 2 wk (17). In these individuals, the total urinary excretion ranged from 1% to 45% of the administered dose. Although full analysis of these results is not yet available, it is already clear that successive measurements of the degree of conversion on 4 days during the experimental period were much more consistent within individuals than between individuals. This result suggests that intrinsic and extrinsic individual factors are important determinants of the degree of conversion of glucosinolates to isothiocyanates.

Similarly, in a study with three healthy human subjects who received a single dose of broccoli sprout glucosinolate preparation (144 µmol), the total urinary excretion of DTCs ranged from 2.5% to 19% of the administered dose (4). In addition, in studies with glucosinolates derived from watercress, Getahun and Chung showed that conversion to DTC ranged from 17% to 78% of the dose for uncooked (containing active plant myrosinase) and from 1% to 7% for cooked (inactivated myrosinase) vegetable (24). Notably, in the present study, the mean cumulative excretion was 412 µmol for the high dose and 93.6 µmol for the low dose. This 4.4-fold difference corresponds to the 4-fold difference in dose, suggesting that the capacity of the gastrointestinal flora to convert glucosinolates to isothiocyanates was not exceeded and that probably even higher doses of glucosinolates could be converted to isothiocyanates with similar efficiencies.

In contrast, the fractions of dose excreted in three individuals who received 25 µmol of the isothiocyanate preparation (525 µmol total dose) were 68.9%, 70.1%, and 72.9% (mean = 70.5 ± 2.0%). Excretion of isothiocyanate DTC metabolite(s) is therefore extremely constant among individuals and is a much higher fraction of the dose than for glucosinolates. In earlier studies (15), four subjects received single oral doses of 200 µmol of isothiocyanates in the form of a similar broccoli sprout extract and excreted 117 ± 6.18 µmol of DTC in 8 h (58.3 ± 6.18%). The slightly lower fraction excreted is probably attributable to the fact that the excretion is incomplete in the 8-h collection period used in the earlier study. We conclude that, in contrast to the conversion of glucosinolates, the pharmacokinetics of the isothiocyanate preparation are rather similar in human subjects and probably not significantly influenced by differences among individuals.

Safety Endpoints

All subjects were questioned daily in an uncoached, nondirected manner about symptoms possibly related to administration of the test substances or placebo. Responses were elicited by an independent observer who was not involved in the study and was blinded with respect to the treatment group of the subjects. Reports of this observer where analyzed before breaking the code and revealed no significant or persistent symptomatic abnormalities at any time point (25).

Routine blood and urine analyses as well as more specialized tests (thyroid and liver function) were obtained. In the absence of indications of specific toxicities, attention was directed to the thyroid and liver because toxicities to these organs have been observed in animals receiving certain glucosinolates (indole and hydroxybutenyl glucosinolates), even though they are not present in significant amounts in broccoli sprouts (6). If subjects showed normal blood tests at the time of screening, they were admitted to the General Clinical Research Unit at Johns Hopkins Hospital. All values outside normal limits are recorded in Table 1. If we analyze all 12 subjects (treated and placebo) as a group, no abnormal values were observed at any time point in 15 of the 32 blood tests. Of the 68 abnormal values in the other 17 blood tests, most were only slightly outside normal limits, and 17 occurred prior to treatment (of which 12 were in the placebo subjects) and sometimes persisted during the entire experimental period. Of the 51 abnormal tests that occurred after initiation of treatment, 14 were observed in subjects receiving placebos. The results of the blood tests were compiled prior to breaking the treatment code.

Only two endpoints showed changes that required further consideration: liver and thyroid function test abnormalities (Table 1).

Liver function tests: Plasma ALT levels rose during the course of the study for 11 of the 12 subjects; in 9 of them, the increase began before dosing (Fig. 3). Levels exceeded the upper limits of normal on one occasion for one individual and on three occasions for a second individual (Table 1). The former does not meet the criterion for a clinically significant adverse event; the latter is classified as grade 1 toxicity (23). Both of these subjects received active preparations. Anecdotal experience at Johns Hopkins and elsewhere indicates that healthy subjects who participate in in-patient phase I drug safety studies often develop mild transaminase eleva-

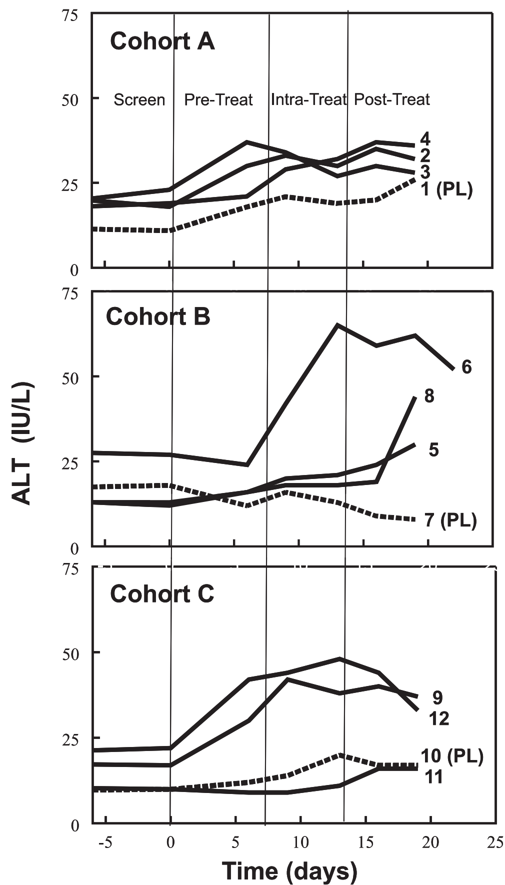

Figure 3. Serum alanine transaminase (ALT) values (expressed as International Units per milliliter) for all 12 subjects (cohort A, upper; cohort B, middle; cohort C, lower panel). The ALT activities are shown at the time of screening, on Days 0 and 6 (pretreatment), Days 9 and 13 (intratreatment), and Days 16 and 19 (posttreatment).

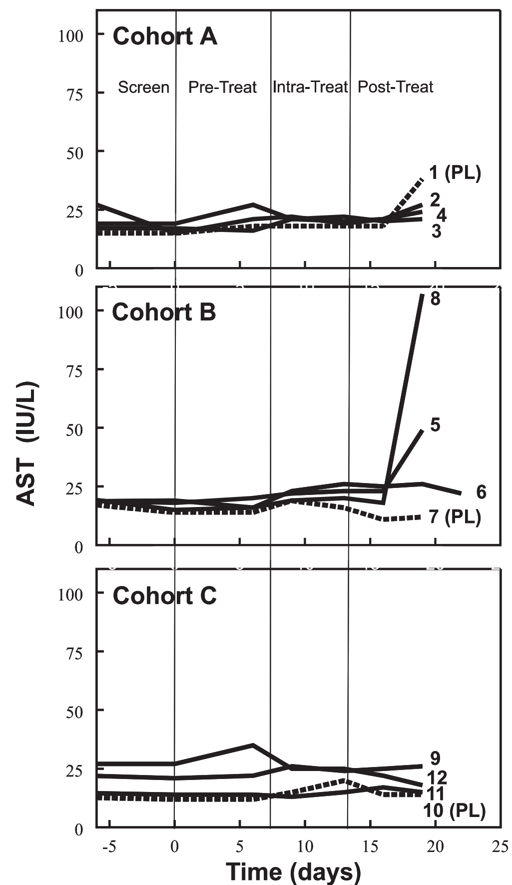

Figure 4. Serum aspartate transaminase (AST) values (expressed as International Units per milliliter) for all 12 subjects (cohort A, upper; cohort B, middle; cohort C, lower panel). The AST activities are shown at the time of screening, on Days 0 and 6 (pretreatment), Days 9 and 13 (intratreatment), and Days 16 and 19 (posttreatment).

tions, attributable to hepatic glycogen storage that occurs with generous meals and relative inactivity. That ALT rises in 11 of the 12 subjects, including placebo recipients, supports this notion. A rise in AST (Fig. 4 and Table 1) above normal on Day 19 occurred in two subjects (both treated with a high dose of glucoraphanin). These subjects were released from the hospital on Day 17, and their postdischarge behavior (for example, possible alcohol consumption) could not be ascertained.

Thyroid function tests: Although not statistically significant, there were notable changes in TSH not associated with any clinical signs or symptoms and not accompanied by abnormalities in total triiodothyronine (T3) or free thyroxine (T4) (Table 1 and Fig. 5). For 11 of the 12 subjects, values of TSH rose during the first 6 days of hospitalization, before dosing with broccoli sprout extracts was begun (Fig. 5). These values remained within the upper limits of normal. In three subjects, TSH levels exceeded the upper limits of normal during or after the dosing period (normal = 0.4–4.2; observed = 4.4–7.6 microIU/ml). Although treatment codes were still masked from investigators, these results were evaluated by two independent experts on the endocrinology of the thyroid gland who had not been involved with this study. Both noted that the changes were mild and reversible and posed no obstacle to further studies with broccoli sprout extracts. *After* breaking the code, it was determined that one of the three subjects with elevations of TSH was a placebo recipient.

In summary, an extensive, double-blind, placebo-controlled, randomized clinical study of the safety and tolerance of repeated doses of broccoli sprout extracts (calibrated to contain precise levels of either glucosinolates or isothiocyanates) in healthy volunteers revealed no evidence of systematic, clinically significant, adverse effects that could be attributed to the sprout extract administration.

Acknowledgments and Notes

P. Talalay, J.W. Fahey, and Johns Hopkins University are founders, unpaid consultants, and equity holders in Brassica Protection Products LLC (BPP), a company that is licensed by Johns Hopkins University to produce broccoli sprouts. These parties may be entitled to royalty payments, and their equity interest in BPP is managed according to university policies. P. Talalay's son is the CEO of BPP. A portion of the proceeds of BPP is used to support cancer research, but no funds were provided to support this study.

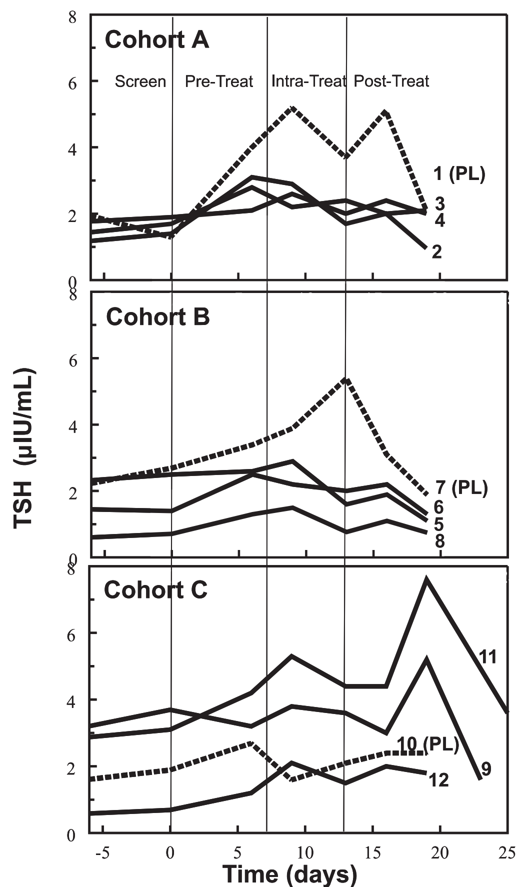

Figure 5. Serum thyroid-stimulating hormone (TSH) values (expressed as micro–International Units per milliliter) for all 12 subjects (cohort A, upper; cohort B, middle; cohort C, lower panel). The TSH values are shown at the time of screening, on Days 0 and 6 (pretreatment), Days 9 and 13 (intratreatment), and Days 16 and 19 (posttreatment). Additional later determinations were made on subjects 9 and 11.

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