In Vivo Production and Enzyme-Inducing Activity of Indolo[3,2-*b*]carbazole

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Indolo[3,2-*b*]carbazole (ICZ) is a potent Ah receptor agonist produced during the oligomerization of indole-3-carbinol (I3C), a breakdown product of the glucobrassicin present in food plants of the *Brassica* genus. Levels of ICZ present in the feces, urine, gastrointestinal tracts, and livers of rats fed on I3C- or cabbage-supplemented basal diet were 16-fold to over 100-fold higher than levels for animals on the basal diet alone. Levels of ICZ significantly lower than the basal levels for conventional rats were present in feces of germfree rats, indicating that gut bacteria are important for the production of ICZ from essential dietary constituents. Low levels of ICZ in extracts of human feces were also detected. The results suggest further that ICZ by itself may not be responsible for the enzyme-inducing activity of orally administered I3C or its precursors.

Keywords: *Cytochrome P450; Brassica; indole-3-carbinol; indolo[3,2-b]carbazole; endogenous inducer*

INTRODUCTION

Indolo[3,2-*b*]carbazole (ICZ) (Figure 1) is a polycyclic, aromatic amine the biological activity of which is similar in several respects to that of the potent environmental toxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). ICZ is nearly isosteric with TCDD, and both substances bind with high affinity to the Ah receptor (Gilner et al., 1985; Bjeldanes et al., 1991). The Ah receptor mediates the transcriptional activation of several genes involved in the regulation of cell growth or the metabolism of steroid hormones and certain carcinogens. The toxicity of TCDD is thought to depend on its binding to the Ah receptor. Whereas both ICZ and TCDD induce cytochrome P4501A1 (CYP1A1)-dependent monooxygenase activity in murine hepatoma cells (Bjeldanes et al., 1991) and both compounds are immunotoxic, as indicated by production of reduced lymphoid development in murine fetal thymus organ culture (d'Argy et al., 1989), ICZ is approximately 10^{-3}–10^{-4} as potent as TCDD with respect to these activities.

We have shown in previous studies that ICZ is produced in vitro and in vivo from the natural plant metabolite indole-3-carbinol (I3C) (Bjeldanes et al., 1991). I3C is an autolytic breakdown product of the glucosinolate glucobrassicin present in *Brassica* plants such as cabbage, kale, and Brussels sprouts. I3C is readily converted under mild aqueous conditions into a series of cyclic and acylic oligomeric products including ICZ (Grose and Bjeldanes, 1992). We observed that compared to control animals treated with the corn oil vehicle only, ICZ and several other indolylic oligomers were produced in considerably greater quantities in the lower gastrointestinal tract and feces of rats treated by oral intubation with I3C. Our results also suggested that ICZ was present in the feces of the control animals not treated with I3C or another known precursor of ICZ.

For the present study we examined more closely the production and biological activity of ICZ in vivo. Our findings indicated that incorporation of either homogenized or whole freeze-dried cabbage in the feed led to large increases in the levels of ICZ in the feces and lower gastrointestinal tract of rats. We observed that while ICZ is readily detectable in the feces of conventional rats fed on a purified diet and in human feces, levels of ICZ in the feces of germfree animals fed on the basal diet were significantly reduced and at the limits of detection. We also show that while ICZ is a strong inducer of hepatic CYP1A1 activity, the levels of ICZ in livers 20

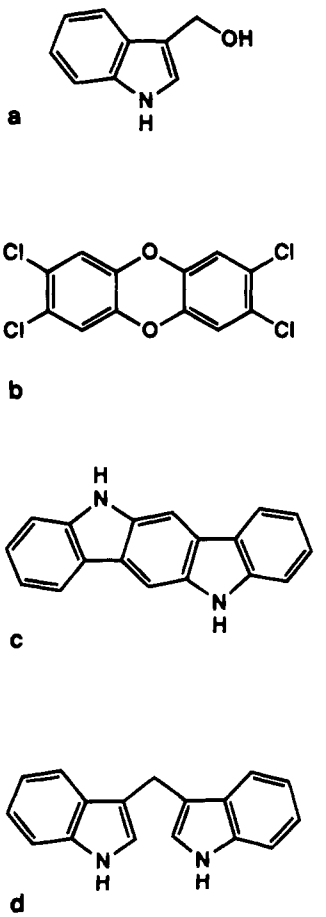

Figure 1. (a) Indole-3-carbinol (I3C); (b) TCDD; (c) indolo[3,2-*b*]carbazole (ICZ); (d) 3,3′-diindolylmethane.

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Table 1. Composition of Experimental Diets

component	concn (g/kg) in diet		component	concn (g/kg) in diet	
	basal	25% (w/w) cabbage		basal	25% (w/w) cabbage
casein	200	150	mineral mix[a]	35	35
DL-methionine	3	3	vitamin mix[a]	10	10
cornstarch	150	100	choline bitartrate	2	2
sucrose	500	370	cellulose	50	30
corn oil	50	50	savoy cabbage (dried)	0	250

[a] Mineral mix and vitamin mix were according to the AIN 76 (Bieri et al., 1977) diet for the rat.

h after I3C treatment appear to be too low to account for the induced activity.

EXPERIMENTAL PROCEDURES

Chemicals. DEAE-Sephadex A-25 buffer reagents were purchased from Sigma Biochemicals (St. Louis, MO) and indole-3-carbinol (I3C) and resorufin from Aldrich Chemical Co. (Milwaukee, WI). Ethoxyresorufin was purchased (ICN Biochemicals, Cleveland, OH) and used directly or was synthesized and purified as described by Mayer et al. (1977). I3C was purified by recrystallization from toluene to yield colorless, opalescent flakes. ICZ was prepared and recrystallized according to the procedure of Robinson (1963). An authentic sample of glucobrassicin was kindly provided by G. R. Fenwick (AFRC Institute of Food Research, Norwich, England). Ethyl acetate for extraction was of HPLC grade. All HPLC grade solvents and water were purchased from Fisher Scientific (San Francisco, CA). As part of a rigorous cleaning procedure to eliminate ICZ contamination, we treated the glassware used in extractions for about 1 h with freshly prepared Nochromix–sulfuric acid solutions (Godax Laboratories, Inc., New York, NY).

A. Studies with Rats. *Animals and Diets.* We purchased male Sprague-Dawley rats (weighing 140 ± 5 g) from Simonsen's Lab (Gilroy, CA) and housed them individually in stainless steel cages with a room temperature of 22 ± 2 °C and with a 12-h light/dark cycle. We equilibrated rats on a semipurified diet containing AIN-76 vitamins and mineral mix (Bieri et al., 1977) (basal diet, Table 1) for 7 days and then randomized them by weight and assigned them to one of four treatment groups. For the preparation of the cabbage diets, we purchased savoy cabbage at a local supermarket and quartered and collected the outer leaves. We mixed these thoroughly and divided them into two batches. One batch, termed "fresh", was frozen at −70 °C and freeze-dried. A second batch, termed "homogenized", was homogenized to a thick slurry (using a Polytron homogenizer; Brinkman Instruments, Westbury, NY) prior to deep-freezing and freeze-drying. We incorporated each cabbage preparation as 25% by dry weight of the semipurified diet. The savoy cabbage diet was prepared by substituting the freeze-dried, powdered vegetable for an equal amount of sucrose, cornstarch, fiber, and protein proportional to the plant's proximate analysis (Table 1). We dissolved recrystallized I3C in corn oil containing 10% DMSO (by volume) and administered it orally in a total volume of 100 μL at a dose of 73.5 mg (500 μmol)/kg of body weight. In a separate experiment, we dissolved ICZ in 10% (by volume) DMSO in corn oil, and we administered by oral intubation or intraperitoneal injection doses of 0.05, 5.0, or 3.0 μmol/kg of body weight in 100 μL total volume. Control rats received 100 μL of 10% DMSO (by volume) in corn oil. Water and experimental diets were provided *ad libitum* for 5 days, and feed was removed following treatment with I3C, ICZ, or corn oil. Twenty hours after dosing, we euthanized the animals with CO_2 and then excised their liver, lungs, upper small intestine (proximate 30 cm), and gastrointestinal contents and tracts. We collected fecal and urine samples during the 5 days of feeding, froze them immediately on dry ice, and stored them at −70 °C. For the ICZ experiment we collected only livers and lungs.

Preparation of Tissues. The contents of the gastrointestinal tract were obtained by flushing with saline as follows: Gastric content (in 5 mL), upper (proximal 30 cm) and lower small intestinal content (in 20 mL each), and colonic and cecal content (in 10 mL each) were perfused with 0.9% NaCl. We split the upper small intestine up the mesenteric artery and laid it flat on a metal plate, and we removed the mucosa by lightly scraping the exposed lumenal wall three times with a metal spatula. The mucosal scrapings were pooled in 1 mL of ice-cold buffer A (0.1 M sodium phosphate, 0.25 mM phenylmethanesulfonyl fluoride, 1 mM dithiothreitol, pH 7.4). Collected samples were immediately frozen on dry ice and stored at −70 °C.

Preparation of Microsomes. We homogenized livers and lungs in 4 volumes of ice-cold potassium phosphate buffer (0.1 M potassium phosphate, 0.25 M sucrose, pH 7.4) using a Polytron homogenizer. Mucosa of the upper small intestine was homogenized in 3 mL of ice-cold buffer A. Liver, lung, and mucosal homogenates were centrifuged at 9000*g* for 10 min at 4 °C, followed by centrifugation of the postmitochondrial fraction for 60 min at 105000*g* (0–4 °C). We resuspended the precipitated microsomal pellet of liver and lung in 3 mL of ice-cold potassium phosphate buffer. The microsomal pellet of mucosa was suspended in 2 mL of ice-cold buffer B (0.1 M potassium phosphate, 0.25 M sucrose, 1 mM dithiothreitol, pH 7.4), frozen immediately on dry ice, and stored at −70 °C for no more than 2 months prior to analysis.

Enzyme Assays. We determined microsomal ethoxyresorufin *O*-deethylase (EROD) activity according to a modification of the method of Burke et al. (1974). In this assay hepatic (100–200 μg), pulmonary (100–200 μg), or small intestinal (300–600 μg) microsomal protein was added to 1.4 mL of 0.1 M potassium phosphate buffer (pH 7.8), followed by the stepwise addition of 15 μL of 50 μM ethoxyresorufin in ethanol and 7.5 μL of 0.05 M NADPH. We determined the rate of formation of resorufin using a Perkin-Elmer 650-10w spectrofluorometer (Perkin-Elmer, Norwalk, NJ) with excitation at 510 nm, emission at 586 nm and a slit width of 10 nm. Protein concentrations were measured according to the method of Bradford (1976) using bovine serum albumin as a standard.

Extraction and HPLC Analysis of Glucobrassicin from Savoy Cabbage. We used a modification of the method described by Minchinton et al. (1982). Approximately 1 g of freeze-dried powdered savoy cabbage was boiled with 100 mL of methanol for 3 min. The resulting suspension was filtered through Whatman No. 1 filter paper, and the residue was re-extracted for 3 min with 100 mL of boiling 75% (v/v) methanol in water. The filtrates were combined, and the methanol was removed in vacuo at 35 °C. The aqueous solution was transferred quantitatively to a volumetric flask. We added 5 mL of 0.5 M barium/lead acetate solution and adjusted the volume to 50 mL with water. The solution was centrifuged at 1000*g* for 10 min, and 5 mL of the supernatant was diluted to a final acetate concentration of 0.025 M. The diluted extract was then applied to columns of DEAE-Sephadex A-25 (100 mg) and desulfated on the columns with arylsulfatase. After overnight reaction with the sulfatase at ambient temperature, the desulfoglucosinolates were eluted into a 5-mL volumetric flask with 4 × 1 mL of water. We adjusted the volume to 5 mL and performed the HPLC analysis of glucobrassicin at room temperature on a Beckman Model 332 HPLC (Beckman Instruments, Fullerton, CA) fitted with an Ultrasphere ODS [4.6 (i.d.) × 250 mm] column (particle size 5 μm; Beckman, San Ramon, CA) using a wavelength setting of 229 nm (Model 160 UV detector; Beckman Instruments). Glucobrassicin was eluted with a reversed-phase gradient of acetonitrile in 31 mM ammonium phosphate buffer (pH 6.7). The solvent program

was 100% ammonium phosphate buffer for 10 min, 0–20% acetonitrile over the next 30 min, and then constant 20% acetonitrile for 20 min with a solvent flow rate of 1.0 mL/min. We quantified glucobrassicin by comparison of peak areas with external standards.

Extraction and HPLC Analysis of I3C from Savoy Cabbage. We extracted freeze-dried, powdered savoy cabbage (1 g) 3 × 10 min with 30 mL of acetonitrile using an electric shaker. The acetonitrile extract was filtered through Whatman filter paper (No. 41), and the filtrate was then evaporated in vacuo at 22 °C. The residue was dissolved in 1.6 mL of acetonitrile, and 0.4 mL of 5 mM ammonium phosphate buffer (pH 7.0) was added. A 1.0-mL aliquot was loaded onto a sample cleanup column (Spice C_{18} sample preparation cartridge; Analtech Inc., Newark, DE) previously equilibrated with 8:2 (v/v) acetonitrile–ammonium phosphate buffer. We performed HPLC analysis of I3C at room temperature with an Ultrasphere ODS column using a wavelength setting of 280 nm. I3C was eluted in a reversed-phase gradient system, with acetonitrile in 5 mM ammonium phosphate buffer (pH 7.0) increasing from 30 to 50% over 40 min (solvent flow rate of 1.0 mL/min). I3C was quantified by comparison of peak areas with external standards.

Extraction and HPLC Analysis of ICZ from Savoy Cabbage, Gastrointestinal Contents, Feces, and Urine of Conventional Rats. We extracted freeze-dried powdered savoy cabbage (1 g) and liver homogenate (5 mL) for 10 min with 30 mL of ethyl acetate (HPLC grade) using an electric shaker. Gastrointestinal contents were homogenized with a Polytron homogenizer for 30 s and extracted with 30 mL of ethyl acetate. In the experiment with normal rats, feces were weighed, swollen with a 10-fold volume of distilled water, and then extracted with 100 mL of ethyl acetate. Urine samples were filtered through Whatman No. 1 paper and extracted for 10 min each with 3 × 50-mL portions of ethyl acetate using an electric shaker. The organic layers were pooled and evaporated in vacuo at 35 °C. The residue was redissolved in 3 mL of 57% (v/v) acetonitrile in 31 mM ammonium phosphate buffer (pH 6.7), filtered through a nylon membrane (0.45 μm), and diluted with the same solvent to an appropriate concentration for analysis by HPLC. ICZ was analyzed by HPLC using a C_{18} bonded-phase column with 57% (v/v) acetonitrile in ammonium phosphate buffer as the mobile phase. We estimated the amount of ICZ in each sample using a fluorescence detector with emission at 415 nm and excitation at 335 nm. Recovery of ICZ from spiked samples was in the 25–75% range.

Analysis of ICZ in Feces from Germfree Rats. The rearing of germfree rats and fecal collection were conducted by Taconic Laboratories (Germantown, NY). Male Sprague-Dawley rats were housed under sterile conditions from birth and at weaning given free access to water and NIH-31 feed. At 6 weeks of age, five rats were transferred to a dedicated study isolator and started on the AIN-76 diet. After 1 week on this diet, fecal collections were begun and continued for 7 days. Specimens from each rat were pooled daily and frozen at −20 °C. Following 21 days of collection, samples were shipped on dry ice to our laboratory, where samples from each rat were pooled, freeze-dried, and ground to a powder. A group of three conventional rats of the same sex, strain, and age as their germfree counterparts and receiving the AIN-76 diet served as the controls. We freeze-dried and assayed the fecal samples for ICZ according to the method described for human samples.

B. Analyses of Human Fecal Samples. *Subjects and Diets.* We obtained the freeze-dried fecal samples of subjects eating controlled diets in an unrelated study (these were kindly provided by the laboratory of J. C. King, University of California, Berkeley, CA). One sample (HFA) was the product of pooling several individual samples. The second sample (HFB) was from a single individual. A dietary record for the HFB sample indicated that for the 3 days immediately prior to the sample collection no more than one serving of *Brassica* vegetables was consumed.

Extraction of ICZ. We extracted the freeze-dried and powdered fecal samples (typically 5 g) with 5 × 50-mL portions of ethyl acetate (HPLC grade). For each of the five portions of solvent, the sample was stirred for at least 1 h at room temperature before we filtered off the insoluble residues which were then re-extracted with the next solvent portion. We pooled the collected filtrates and removed the ethyl acetate in vacuo at about 35 °C. We transferred the sample residues into 4-mL vials using ethyl acetate, and then applied a 50-μL portion of this redissolved solution to a dry sample-preparation cartridge packed with silica gel (SPICE Si, Analtech, Newark, DE). We then applied three portions of eluting solvent to obtain the ICZ-containing fraction: 2 mL each of hexanes, 5% ethyl acetate in hexanes, and finally 20% ethyl acetate in hexanes (all percentages are v/v). The third 2-mL fraction was collected and evaporated to dryness under nitrogen with heating at 35 °C and then redissolved in 50 μL of acetonitrile for injection and analysis by HPLC.

HPLC Methods. For all of the HPLC analyses we used a C_{18} bonded-phase column [Beckman Ultrasphere ODS, 0.46 (i.d.) × 25 cm]. The mobile phase solution was 57% (v/v) acetonitrile in 31 mM ammonium phosphate adjusted to pH 6.7 with aqueous ammonia. The sample volumes were 10 μL, and the column flow rate was 1 mL/min. The detector was a Perkin-Elmer Model LS-4 fluorescence spectrometer set for excitation at 335 nm and emission at 415 nm.

Statistics. For Table 3, two-way analyses of variance of untransformed data were performed, and for Tables 2 and 4, analysis of variance of log-transformed data was performed. When found to be significant, the analyses were followed with Tukey's Studentized range test at a procedure-wise error rate of 5%.

RESULTS AND DISCUSSION

ICZ Levels in Liver, Gastrointestinal Contents, Feces, and Urine of Rats Fed I3C or Savoy Cabbage. We were unable to detect ICZ in liver, small intestinal content, or urine of rats fed on the basal diet (Table 2). However, we detected ICZ in the contents of stomach, colon, and cecum and in the feces of these control animals. ICZ levels were markedly increased in the tissues and excreta of animals either treated with I3C or fed on diets supplemented with freeze-dried savoy cabbage containing glucobrassicin at 470 mg/100 g and with ICZ levels below 0.5 μg/100 g. The levels of ICZ for tissues or excreta were not significantly different for the three treatment groups. The relative increases in ICZ levels for the treated groups, when compared to samples in which basal levels were detected, ranged from approximately 16-fold for the cecum to about 60-fold for the stomach. The lowest concentrations of ICZ in samples from treated rats were found in urine and the highest concentrations in feces.

Analyses of ICZ in Feces of Germfree Rats and Humans. In a separate experiment in which we determined the ICZ content in the feces of germfree and conventional rats fed on AIN-76 diets, we observed that whereas ICZ levels in the feces of conventional rats were 2.00 ± 0.50 ppb (w/w), levels from germfree animals were around 0.40 ± 0.20 ppb, both figures being based on the wet weight of feces. This difference was statistically significant at $p < 0.04$.

Over many repeated analyses, the HPLC chromatograms of the products extracted from the two human fecal samples consistently included a peak for ICZ. This peak was equivalent to an ICZ level in the range of 2–20 ppb (w/w) in the original samples, based on dry weights and allowing for uncertainties in recovery and other experimental variation.

Further evidence that ICZ was present in these human and basal rat samples is as follows: (*i*) no ICZ peak was detected in blank extractions performed with the same volumes of extracting solvents and with rigorously cleaned glassware; (*ii*) the peak measured in the chromatograms of the extraction samples had the

Table 2. Effect of Indole-3-carbinol (I3C) and Dietary Savoy Cabbage on the Amount[a] of Indolo[3,2-*b*]carbazole (ICZ) in Liver, Gastrointestinal Contents, Feces, and Urine of Rats

	ICZ (pg/total sample)			
diet	liver	gastric content	upper small intestinal content	lower small intestinal content
basal	$0.0^{a} \pm 0.0$	$9.7^{a} \pm 2.9$ (24.3 ± 7.3)[b]	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$
basal–I3C	$116.9^{b} \pm 45.6$ (14.8 ± 5.7)	$808.5^{b} \pm 419.5$ (898.3 ± 466.1)	$25.4^{b} \pm 19.3$	$180.4^{b} \pm 91.9$
25% fresh savoy	$45.2^{b} \pm 12.1$ (5.5 ± 1.5)	$656.2^{b} \pm 551.9$ (656.2 ± 551.9)	$17.0^{b} \pm 3.7$	$310.1^{b} \pm 187.7$
25% homogenized savoy	$70.9^{b} \pm 11.5$ (8.1 ± 1.3)	$423.7^{b} \pm 210.7$ (184.2 ± 91.6)	$23.7^{b} \pm 17.5$	$453.8^{b} \pm 151.0$

	ICZ (pg/total sample)			
diet	colonic content	cecal content	feces	urine
basal	$18.3^{a} \pm 6.0$	$38.6^{a} \pm 11.8$ (12.1 ± 3.7)	$382.4^{a} \pm 164.0$ (88.9 ± 38.1)	$0.0^{a} \pm 0.0$
basal–I3C	$682.8^{b} \pm 234.0$	$590.5^{b} \pm 116.3$ (164.0 ± 32.3)	$13681.4^{b} \pm 8284.9$ (3508.1 ± 2124.4)	$0.9^{a} \pm 0.6$ (0.0 ± 0.0)
25% fresh savoy	$616.9^{b} \pm 158.1$	$621.2^{b} \pm 59.2$ (151.5 ± 14.4)	$23323.4^{b} \pm 9022.5$ (4573.2 ± 1769)	$22.0^{b} \pm 3.6$ (1.5 ± 0.2)
25% homogenized savoy	$744.8^{b} \pm 63.8$	$725.2^{b} \pm 83.0$ (181.3 ± 20.8)	$15984.5^{b} \pm 5352.4$ (3262.1 ± 1092.3)	$49.4^{b} \pm 17.5$ (1.5 ± 0.5)

[a] Values are mean ± SE for groups of four rats. Those values in a column not sharing a common superscript are significantly different from one another ($p < 0.05$). Rats were fed basal diet or basal diet plus 25% (w/w) savoy cabbage for 5 days. I3C was dissolved in 100 μL of corn oil containing 10% DMSO (v/v) and administered by oral intubation (500 μmol/kg of body wt). [b] Values in parentheses are in units of pg/g of sample.

Table 3. Effect of Indole-3-carbinol (I3C) and Dietary Savoy Cabbage on Ethoxyresorufin *O*-Deethylase (EROD) Activity[a] in Liver, Small Intestinal Mucosa, and Lungs of Rats

	EROD [pmol of resorufin formed min^{-1} (mg of protein)$^{-1}$]		
diet	liver	small intestine	lungs
basal	$4.4^{a} \pm 0.6$	$0.4^{a} \pm 0.2$	$0.1^{a} \pm 0.0$
basal–I3C	$164.0^{b} \pm 63.5$ (37)	$13.1^{b} \pm 5.3$ (33)	$3.1^{b} \pm 0.7$ (31)
25% fresh savoy	$19.5^{c} \pm 6.2$ (4.4)	$37.2^{b} \pm 27.0$ (93)	$2.2^{b} \pm 1.3$ (22)
25% homogenized savoy	$15.2^{c} \pm 2.8$ (3.5)	$15.3^{b} \pm 8.5$ (38)	$1.1^{a,b} \pm 0.5$ (11)

[a] Values are mean ± SE for groups of four rats. Figures in parentheses represent activity relative to the basal group. Those values in a column not sharing a superscript are significantly different ($p < 0.05$). Rats were fed basal diet or basal diet plus 25% (w/w) savoy cabbage for 5 days. I3C was dissolved in 100 μL of corn oil containing 10% DMSO (v/v) and administered by oral intubation (500 μmol/kg of body wt).

same retention time and peak shape as did authentic ICZ under a range of HPLC elution conditions; (*iii*) the analysis of chromatograms obtained by spiking the extracted sample with authentic ICZ showed an appropriate increase in the suspected ICZ peak area, and no additional peaks were produced; (*iv*) the fluorescence emission and excitation spectra of the suspected ICZ peak (obtained from the extraction of sample HFA scaled up by 100-fold) were identical to those of the authentic compound.

The most likely source of ICZ in the feces of control animals and humans is bacterial metabolism of tryptophan. Skatole and indole, as well as unidentified ligands for the Ah receptor, are established breakdown products of tryptophan (Brown, 1977; Perdew and Babbs, 1991). Several oxidized derivatives including 3-methyloxindole, indole-3-carboxylic acid, and I3C are established mammalian metabolites of skatole (Skiles et al., 1989). Conversion of these or similar products to ICZ in the gut is a likely possibility.

Induction of EROD Activity in Rats Fed Savoy Cabbage or Treated with I3C or ICZ. None of the rats in the control or experimental groups showed adverse affects from their treatments. No significant differences in weight gains (6.56 ± 1.11 g) (mean ± SE), feed intakes (69.88 ± 1.92 g), or liver weights (8.45 ± 0.19 g) were observed among the groups. In rats that received a single treatment of I3C [500 μmol/kg of body weight dissolved in 100 μL of corn oil containing 10% DMSO (v/v)] administered by oral intubation, there were significant increases in EROD activities in the liver, small intestinal mucosa, and lungs of approximately 37-, 33-, and 31-fold, respectively, over the corn oil-treated controls (Table 3). EROD activities in the livers of rats fed either fresh or homogenized savoy cabbage as 25% (w/w) of the diet for 5 days were about 4-fold higher than in control rats. EROD activities in rats fed on the fresh or homogenized cabbage were strongly induced in the intestinal mucosa (93- and 38-fold, respectively and lungs (23- and 11-fold, respectively). These results are consistent with previous findings (McDanell et al., 1987) and indicate that the EROD-inducing effects of cabbage are not mimicked simply by administration of a corresponding dose of I3C. The role of other possible factors following ingestion of cabbage such as decreased translocation of inducing agents to the liver or the production of inhibitors of EROD activity or CYP1A1 gene expression is indicated by these results.

Treatment of rats with doses of ICZ (i.e., 0.05, 3.0, and 5.0 μmol/kg) that are in the range of doses expected to result from I3C in vivo (Bjeldanes et al., 1991) produced 10–20-fold increases in EROD activities in the liver and lungs of the rats (Table 4). These results indicate that, compared to TCDD, ICZ is a weak inducer of hepatic EROD activity in vivo. Published data indicate that a dose of TCDD of around 60 pmol/kg of body weight produced a 10-fold increase in hepatic

Table 4. Effect of ICZ Treatment on EROD Activity[a] in Rat Liver and Lungs

	EROD activity[c]	
treatment[b]	liver	lung
vehicle[d]	7.1 ± 0.5^{a}	0.2 ± 0.04^{a}
ICZ, 0.05[d]	84.1 ± 13.0^{b} (11.8)	2.1 ± 0.5^{bc} (9.3)
ICZ, 5.0[d]	121.0 ± 20.4^{bc} (17.0)	1.7 ± 0.5^{b} (7.3)
ICZ, 3.0[e]	159.0 ± 8.2^{c} (22.3)	4.5 ± 0.3^{c} (19.7)

[a] Values are means ± SE with $n = 4$ for the first three groups and $n = 3$ for the last group. Groups sharing different superscripts are significantly different from one another ($p < 0.05$). Values in parentheses indicate fold induction over vehicle treated controls. [b] ICZ doses in units of μmol/kg of body wt. [c] EROD activity in units of pmol of resorufin min^{-1} (mg of protein)$^{-1}$. [d] ICZ administered by oral intubation. [e] ICZ administered by intraperitoneal injection.

EROD activity in rats (Abraham et al., 1988). This dose range is only about 0.1% the dose of ICZ found in the present study to produce a 10-fold increase in hepatic EROD activity. This large difference in activities of the two compounds in vivo is consistent with the large differences reported previously for the two compounds in CYP1A1-inducing activities in cell culture (Bjeldanes et al., 1991) and in immunotoxicities in thymus organ culture (d'Argy et al., 1989).

Our results are not consistent with a major role for ICZ in the CYP1A1-inducing effects of I3C. Although ICZ levels tended to be highest in the livers of I3C-treated rats, which also had the highest levels of EROD induction (compare data in Tables 2 and 3), the quantities of ICZ that we detected in the livers appear to be several orders of magnitude too low to produce the observed increases in EROD activity. Again based on published data (Abraham et al., 1988), a tissue level of about 600 fmol/g of TCDD accompanies a 40-fold induction of EROD activity. We determined a level of ICZ in livers of I3C-treated rats of only about 60 fmol/g, or about 10% of the TCDD value. Since ICZ is considerably less active than TCDD in several bioassays, this low tissue level of ICZ is unlikely to have a major effect on the induction of EROD activity. This result suggests that ICZ may serve as a marker for the presence of other I3C derivatives such as diindolylmethane, which, compared to ICZ, is a weak inducer of EROD activity but is produced in much larger quantities, in vitro and in vivo (Grose and Bjeldanes, 1992).

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