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Effects of Biologically Active Substances in Natural Products on the Hepatic Detoxication Mechanism

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

Kwon, Chong-Suk · Grose, Karl R.* · Riby, Jacques*
Chen, Yue-Hwa* · Bjeldanes, Leonard F.*

Department of Food and Nutrition, Andong National University
Department of Nutritional Sciences, University of California, Berkeley, U.S.A*

ABSTRACT

Indolo[3, 2-*b*]carbazole(ICZ) is a potent Ah receptor agonist with biological activities similar in several respects to those of the potent environmental toxin, TCDD. ICZ is produced during the oligomerization of indole-3-carbinol(I3C), a breakdown product of the glucobrassicin present in food plants of the *Brassica* genus. In the present study we examined ICZ levels in tissues and excreta of rats treated with I3C or dietary cabbage of established glucobrasicin content, and in feces of conventional and germfree rats fed on a basal diet, and of humans. We also examined the levels of cytochrome P4501A1 induction, as determined by the ethoxyresorufin *o*-deethylase assay, in tissues of animals that received cabbage-supplemented diets, or which were treated with purified I3C or ICZ. Our findings indicated that incorporation of either homogenized or whole freeze-dried cabbage in the feed led to large increases(16−60 fold) in the levels of ICZ in the feces and lower gastrointestinal tract of rats.

We observed that whereas ICZ is readily detectable at about the same levels(2.00± 0.50 ppb) 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 at the limits of detection(0.40± 0.20 ppb), indication that gut bacteria are important for the production of ICZ from essential dietary constituents in the basal diet. We showed that in contrast to the near 7000-fold difference in CYP1A1 inducing potencies of ICZ and TCDD in cells in culture, their inducing potencies differ by only about an order of magnitude in rats. Nonetheless, the levels of ICZ remaining in livers twenty hours after I3C treatment appear too low to account for the induced activity. This result indicates that ICZ may be rapidly cleared from the liver or that substances other than, or in addition to, ICZ may be responsible for the enzyme-inducing activity of orally administered I3C or its precursors.

KEY WORDS : indole-3-carbinol · indolo[3, 2-*b*]carbazole · cytochrome p4501A1 · savoy cabbage.

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Introduction

Indolo[3, 2-*b*]carbazole(ICZ) 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[1]. The Ah receptor mediates the transcriptional activation of several genes in 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 P4501A 1(CYP1A1)-dependent monoxygease activity in murine hepatoma cells[2], and both compounds are immunotoxic as indicated by production of reduced lymphoid development in murine fetal thymus organ culture[3], ICZ is approximately 10^{-4} to 10^{-5} 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)[2]. 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 acyclic oligomeric products including ICZ[4]. We observed that compared to control animals treated with the corn oil vehicle only, ICZ and several other indolylic oligomers where 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 of ICZ in vivo. Our findings indicated that incoporation 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 purified diet and in human feces, levels of ICZ in the feces of germfree animals fed on the basal diet were at the limits of detection. We also show that while ICZ is a potent inducer of hepatic CYP1A1 activity, the levels of ICZ in livers twenty hours after I3C treatment appear too low to account for the induced activity.

Method

Chemicals : DEAE-Sephadex A-25 buffer reagent were purchased from Sigma Biochemicals (St. Louis, MO), 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 in the literature[5]. I3C was purified by recrystallization from toluene to yield colorless, opalescent flakes. ICZ was prepared and recrystallized by the procedure of Robinson[6]. 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 one hour with freshly prepared Nochromix-sulfuric acid solutions(Godax Laboratories, Inc., New York, NY).

Animals and Diets : Male, Sprague-Dawley rats were purchased(weighing 140± 5 g) from Simonsen's Lab(Gilroy, CA) and housed individually in stainless-steel cages with a room temperature of 22± 2℃ and with a 12-hour light/dark cycle. We equilibrated rats on a semipurified diet containing AIN-76 vitamins and mineral mix[7](basal diet, Table 1) for 7 days and then randomized them by weight and assigned them to one of four treatment groups. Savoy cabbage was purchased at a local supermarket and divided into two batches. One batch, termed 'fresh', was frozen at −70℃ 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(Table 1). Recrystallized I3C was dissolved in corn oil containing 10% DMSO (by volume) and administered orally in a total volume of 100 μL at a dose of 73.5mg(500 μmol/kg) body weight. In a separate experiment, we dissolved ICZ in 10% (by volume) DMSO in corn oil, and administered by PO or IP routes doses of 0.05, 5.0 or 3.0 μmol/kg 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, rats were euthanized with CO_2 and then excised liver, lungs, upper small intestine (proximate 30 cm), 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℃. For the ICZ experiment, only livers and lungs were collected.

Table 1. Composition of experimental diets

Component	Concentration(g/kg) in diet		
	Basal	25% (wt/wt) Cabbage	
Casein	200	150	
DL-Methionine	3	3	
Corn starch	150	100	
Sucrose	500	370	
Corn oil	50	50	
Mineral mix*	35	35	
Vitamin mix*	10	10	
Choline bitartrate	2	2	
Cellubolse	50	30	
Savoy cabbage(dried)	0	250	

**Mineral mix* and *Vitamin mix* were according to the AIN 78(1977) diet for the rat

Human subjects and diets : The freeze-dried fecal samples of subjects eating controlled diets in an unrelated study were obtained(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 he HFB sample indicated that for the three days immediately prior to the sample collection no more than one serving of *Brassica* vegetables was consumed.

Preparation of rat 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), colonic and cecal content(in 10 mL each) were perfused with 0.9% NaCl. Intestine was splitted. The upper small intestine up the mesenteric artery was laid flat on a metal plate, and removed the mucosa by lightly scraping the exposed lumenal wall three times with a metal spatula. The mucosal scrapings were pooled in 1 mL ice-cold buffer A(0.1 M sodium phosphate, 0.25 mM phenylmethylsulphonyl fluoride, 1 mM dithiothreitol, pH 7.4). Collected samples were immediately frozen on dry ice and stored at −70℃.

Preparation of rat tissue microsomes : Livers and lungs were homogenized in 4 volumes ice-cold potassium phosphate buffer(0.1 M potasium phosphate, 0.25 M sucrose, pH 7.4) using a Polytron homogrenizer(Brinkman Instruments, Westbury, NY). Mucosa of the upper small intestine was homogenized in 3 mL ice-cold buffer A. Liver, lung and mucosal homogenates were centifuged at 9000×*g* for 10 min at 4℃, followed by centrifugation of the post-mitochondrial fraction for 60 min at 105,000×*g*(0−4℃) then resuspended the precipitated microsomal pellet of liver and lung in 3 mL ice-cold potassium phosphate buffer. The microsomal pellet of mucosa was suspended in 2 mL 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℃ for no more than 2 months prior to analysis.

Enzyme assay : Microsomal ethoxyresorufin *O*-deethylase(EROD) activity were determined by a modification of the method of Burke et al[8].

Extraction & HPLC analysis of glucobrassicin and I3C from Savoy cabbage : For extraction of glucobrassicin, a modified method of Minchinton et al[10] was used. I3C was extracted from freeze-dried, powdered Savoy cabbage with acetonitrile. The acetonitrile extract was filtered through Whatman filter paper(No. 41) and the filtrate was then evaporated in vacuo at 22℃. 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. The HPLC analysis of glucobrassicin and I3C was performed at room temperature on a Beckman Model 332 HPLC(Beckman Instruments, Fullerton, CA) fitted with an Ultrasphere-ODS column using a wavelength setting of 229 nm for glucobrassicin and 280 nm for I3C(Model 160 UV detector ; Beckman Instruments, Fullerton, CA).

Extraction & HPLC analysis of ICZ from Savoy cabbage, gastrintestinal contents, feces and urine of conventional rat and feces from human subjects : ICZ was extracted from freeze-dried powdered Savoy cabbage and liver homogenate and gastrointestinal contents with ethyl acetate(HPLC grade). Feces were weighed and then extracted with ethyl acetate. Urine samples were filtered and extracted for 10 min each 3 times with ethyl acetate. The organic layers were pooled and evaporated in vacuo at 35℃. The residue was redissolved in 3 mL of 57% (v/v) acetonitrile in 31 mM ammonium dihydrogen 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 PHLC using a C-18 bonded-phase column with 57% (v/v) acetonitrile in ammonium dihydrogen 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.

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, 5 rats were transferred to a dedicated study isolator and started on the AIN-76 diet. After one week on this diet, fecal collections were begun and continued for 7 days. Specimens from each rat were pooled daily and frozen at −20℃. 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 3 conventional rats of the same of the same sex, strain, and age as their germfree counterparts and receiving the AIN-76 diet, served as the controls. The fecal samples were freeze-dried and assayed for

ICZ.

Statistics : Results were expressed as the mean ± standard error and analyzed statistically by analysis of variance, followed with Tukey's studentized range test. Significance level for all statistics was set at $P<0.05$.

Results and Discussion

ICZ levels in liver, gastrointestinal contens, 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, 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 levles below 0.5 μg/100g. 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

Table 2. Effect of indole-3-carbinol(I3C) and dietary savoy cabbage on the amount of indolo[3, 2-*b*] carbazole(ICZ) in liver, gastrointestinal contents, feces and urine of rats

Diet	ICZ(pg/total sample)			
	Liver	Gastric content	Upper small intestinal content	Lower small intestinal content
Basal	0.0^{a}± 0.0	9.7^{a}± 2.9 (24.3 ± 7.3)*	0.0^{a}± 0.0	0.0^{a}± 0.0
Basal-I3C	115.9^{b}± 45.6 (14.8 ± 5.7)	808.5^{b}± 419.5 (898.3 ± 466.1)	25.4^{b}± 19.3	180.4^{b}± 91.9
25% Fresh savoy	45.2^{b}± 12.1 (5.5 ± 1.5)	656.2^{b}± 551.9 (656.2 ± 551.9)	17.0^{b}± 3.7	310.1^{b}± 187.7
25% Homogenized savoy	70.9^{b}± 11.5 (8.1 ± 1.3)	423.7^{b}± 210.7 (184.2 ± 91.6)	23.7^{b}± 17.5	453.8^{b}± 151.0

Diet	ICZ(pg/total sample)			
	Colonic content	Cecal content	Feces	Urine
Basal	18.3^{a}± 6.0	38.6^{a}± 11.8 (12.1 ± 3.7)	382.4^{a}± 164.0 (88.9 ± 38.1)	0.0^{a}± 0.0
Basal-I3C	682.8^{b}± 234.0	590.5^{b}± 116.3 (164.0 ± 32.3)	13681.4^{b}± 8284.9 (3508.1 ± 2124.4)	0.9^{a}± 0.6 (0.0 ± 0.0)
25% Fresh savoy	616.9^{b}± 158.1	621.2^{b}± 59.2 (151.5 ± 14.4)	23323.4^{b}± 9022.5 (4573.2 ± 1769)	22.0^{b}± 3.6 (1.5 ± 0.2)
25% Homogenized savoy	744.8^{b}± 63.8	725.2^{b}± 83.0 (181.3 ± 20.8)	15984.5^{b}± 5352.4 (3262.1 ± 1092.3)	49.4^{b}± 17.5 (1.5 ± 0.5)

Values are mean± SE for groups of four rats.

Those values in a column not sharing a common superscript significantly different from one another($p<0.05$).

Rats were fed basal diet or basal plus 25% (wt/wt) Savoy cabbage for 5 days.

I3C was dissolved in 100μL of corn oil containing 10% DMSO(v/v) and administered by oral intubation(500 μmol/kg body wt).

*Values in parentheses are in units of pg/g of sample.

treated rats were found in urine and the highest concentrations were found 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 rat fed on AIN-76 diets, we observed that whereas ICZ levels in the feces of conventional rats were 2.00± 0.50 ppb(wt/wt), levels from germfree animals were around 0.40± 0.20 ppb, both figures based on the wet weight of feces. The levels in the germfree were near the lower limits of detection of our analytical method.

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(wt/wt) 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 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 fluroescence 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 trytophan. Skatole and indole, as wall as unidentified ligands for the Ah receptor, are established breakdown products of tryptophan[11)12)]. Several oxidized derivatives including 3-methyloxindole, indole-3-carboxylic acid, and I3C are established mammalian metabolites of skatole[13)]. 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, feed intakes or liver weights were observed among the groups(Table 3). In rats that received a single

Table 3. Effects of indole-3-carbinol(I3C) and dietary savoy cabbage on body weight, food intake and liver weight of male rats

Diet	Weight gain(g)	Feed intake(g)	Liver weight(g)
Basal	6.24± 0.29*	71.00± 1.25*	8.60± 0.47*
Basal-I3C	9.75± 3.07	75.50± 4.15	8.25± 0.53
25% Fresh savoy	6.50± 2.77	69.25± 3.98	8.276/0.32
25% Homogenized savoy	3.75± 1.19	63.75± 3.98	8.68± 0.27

*Values in each column are not significantly different from one another($p<0.05$)
Values are mena± SE for groups of four rats
Rats were fed basal diet or basal diet plus 25%(wt/wt) savoy cabbage for 5 days
I3C was dissolved in 100μL of corn oil containing 10% DMSO(v/v) and administered by oral intubation(500 μ/kg body wt)

Table 4. Effect of indole-3-carbinol(I3C) and dietary savoy cabbage on ethoxyresorufin *O*-deethylase(EROD) activity in liver, small interstinal mucosa and lungs of rats

Diet	EROD(pmol resorufin formed/min/mg protein)		
	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)	$1.1^{a,b}\pm 0.5(11)$

Values are mean± SE for groyps 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 or basal diet plus 25% (wt/wt) savoy cabbage for 5 days

I3C was dissolved in 100μL of corn oil containing 10% DMSO(v/v) and administered by oral intubation(500 μmol/kg body wt)

treatment of I3C(500 μmol/kg 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 4). EROD activities in the livers of rats fed either fresh or homogenized Savoy cabbage as 25% (wt/wt) of the diet for five days were about 4-fold higher than in control rats. EROD actvities in rats fed on the fresh or homogenized cabbage were strongly induced in the intestinal mucosa(93- and 38-fold) and lungs(23- and 11-fold). These results are consistent with previous findings[14] 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 expressing 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, produced 10-20-fold increases in EROD activities in the liver and lungs of the rats(Table 5). These results indicate that ICZ is a potent inducer of hepatic EROD activity in vivo. Published data indicate that TCDD has an EC_{50} for hepatic EROD induction in rodents on the order of 1-10 nmol/kg[15]. This dose range is only a factor of 5-50-fold less than the dose of ICZ found in the present study to produce a 10-fold increase in hepatic EROD activity. This similarity of activities of the two compounds in vivo is notable in light of the near 7000-fold difference in CYP1A1-inducing activities we observed previously in cells in cultutre.

Table 5. Effect of ICZ treatment of EROD activity in rat liver and lungs

Treatment*	EROD Activity**	
	Liver	Lung
Vehicle(p.o.)	$7.1\pm$ 0.5^{a}	0.2 ± 0.04^{a}
ICZ, 0.05(p.o.)	84.1 ± 13.0^{b}(11.8)	2.1 ± 0.05^{bc}(9.3)
ICZ, 5.0(p.o.)	121.0 ± 20.4^{bc}(17.0)	1.7 ± 0.5^{b}(7.3)
ICZ, 3.0(i.p.)	$159.0\pm$ 8.2^{c}(22.3)	4.5 ± 0.3^{c}(19.7)

*ICZ doses in units of μmol/kg body wt

**EROD activity in units of pmol resorufin/min/mg protein

Values are means± SE with n=4 for the first 3 groups and n=3 for the last group

Groups sharing different superscripts are significantly different from one another($p<0.05$)

Values in parenthesis indicate fold induction over vehicle treated controls

This decreased differece in potency of the two compounds in vivo is likely due to whole body phamacologic processes not present in cells in culture. The results are consistent, for example, with a decreased translocation of TCDD to the liver relative to ICZ, due perhaps to increased association of TCDD with lipid storage sites, compared to the less lipid soluble ICZ.

Our results are only qualitatively consistent with a role for ICZ in the CYP1A1-inducing effects of I3C. ICZ levels are highest in the livers of I3C-treated rats, which also had the highest levels of EROD induction(compare data in Table 2 and 5). However, based on a comparison with published data for TCDD[15], the quantities of ICZ we detected in the livers appear to be at least an order of magnitude too low to produce the observed increase in EROD activity. This result could be explained by a persistence of induced EROD activity with rapid clearance of ICZ from the liver. Alternatively, ICZ may serve as a marker for the presence of other I3C or tryptophan derivatives(such as diindolylmethane) that are weak inducers of EROD activity, but which are produced in much larger quantities than ICZ. A resolution of this issue depends on the application of sensitive assays for I3C products in addition to ICZ.

Conclusions

In conclusion, these data showed either homogenized or freeze-dried savoy cabbage in the feed led to large increases in the ICZ levels in the feces and lower GIT and in the EROD activity of rats. From the analysis of ICZ in feces of germfree rats and humans, gut bacteria are important to produce ICZ from dietary constituent in the basal diet. ICZ may be responsible for the enzyme-inducing activity of orally administered I3C or its precursors.

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= 국문초록 =

천연물중의 생리활성성분이 간해독기구에 미치는 영향

권정숙 · Grose, Karl R.* · Riby, Jacques* · Chen, Yue-Hwa* · Bjeldanes, Leonard F.*
안동대학교 생활과학대학 식품영양학과
Department of Nutritional Sciences, University of California, Berkeley, U.S.A*

Brassica family에 속하는 식물내에 존재하는 glucobrassicin의 분해산물인 indole-3-carbinol(I3C)의 중합으로 생성되는 indolo[3, 2-*b*] carbazole(ICZ)은 환경독성물질인 TCDD와 유사한 생리활성을 가지며, Ah receptor에 대해서도 길항작용을 나타내는 물질이다.

본 연구에서는 Brassica family의 식물중 I3C와 glucobrasicin을 다량함유하고 있는 savoy cabbage를 투여한 동물의 조직과 배설물, basal diet을 투여한 conventional rats와 germfree rats 그리고 사람의 대변에 함유되어 있는 ICZ량을 HPLC로 측정하였으며, 아울러 cabbage, purified I3C 또는 ICZ를 경구투여한 동물조직으로부터 Cytochrome P4501A1(CYPIA1)의 induction 정도를 ethoxyresomfin *O*-deethylase assay로 측정하였다. 그 결과, cabbage를 투여한 동물의 대변과 소장하부에서 ICZ의 량이 다량 증가(16~60배)된 것으로 나타났으며, purified diet을 투여한 conventional rats의 대변과 사람의 대변에서는 ICZ(2.00± 0.50 ppb)가 쉽게 측정이 된 반면 germfree rats의 대변에서는 ICZ(0.40± 0.20 ppb)를 거의 측정할 수 없었다. 이로써 장내 박테리아가 식이내에 함유되어 있는 성분으로부터 ICZ를 생성하는 중요한 역할을 함을 알 수 있었다. 또, ICZ와 TCDD의 CYPIA1 induction 정도는 약 7000배로 세포배양 실험결과 나타났으나, I3C 투여 20시간후 간에 남아 있는 ICZ의 량이 극히 낮은 것으로 봐서 ICZ가 간에서 신속히 제거되거나 또는 ICZ 이외의 어떤 물질이 경구투여한 I3C 또는 그 전구물질들의 enzyme-inducing activity에 관여할 수도 있다고 사료된다.