ORIGINAL

Effects of indole-3-carbinol and phenethyl isothiocyanate on bile and pancreatic juice excretion in rats

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Abstract : Bile and pancreatic juice contain a number of parameters for cancer chemoprevention. Indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC), which are hydrolytic products of brassica plants, have been established to be anti-cancer agents. Here, we developed a method for the continuous and selective sampling of bile and pancreatic juice, and the effects of I3C and PEITC on bile and pancreatic excretion and γ-glutamyl transpeptidase (γ-GTP) activity in the samples were investigated. Male Fisher 344 rats (eight weeks of age) were challenged intragastrically with I3C (150 mg/kg) or PEITC (160 mg/kg) for five days. Twenty-four hours after the final administration, cannulation was undertaken into the rats' bile and pancreatic ducts, and the bile and pancreatic juice were separately collected for 48 h. In this rat model, bile was stably excreted, and the bile and pancreatic excretion of the control rats was 21.9 ± 1.4 ml/48 h and 12.8 ± 1.7 ml/48 h, respectively. Bile excretion for the first 24 h significantly increased in the I3C- or PEITC-treated rats compared with the control rats. In the case of pancreatic juice, excretion during the first 24 h significantly increased in the PEITC-treated rats. In bile, γ-GTP activity was significantly increased for the first 24 h in the I3C- and PEITC-treated rats, but no difference was observed in the pancreatic juice. Increases of bile excretion and γ-GTP activity in bile might be a factor involved in the anti-cancer effect of I3C and PEITC. Our rat model described here is a useful tool for the study of cancer chemoprevention. J. Med. Invest. 59 : 246-252, August, 2012

Keywords **:** *indole-3-carbinol, phenethyl isothiocyanate, bile, pancreatic juice, cancer chemoprevention*

INTRODUCTION

It is well known that cruciferous vegetables contain anti-cancer agents (1). In fact, one epidemiological study has indicated that the consumption of cruciferous vegetables is linked to the reductions in the risks of developing breast, prostate and colon cancer (2). These vegetables, especially cabbage, broccoli, Brussels sprout and cauliflower, are rich in glucosinolate, one of the anti-cancer agents in cruciferous vegetables (3).

Glucosinolate is hydrolyzed by myrosinase (also known as thioglucoside glycohydrolase). Since myrosinase localizes inside plant cells, the cutting and chewing of vegetables release the enzyme and enhance the hydrolysis of glucosinolate. Inversely,

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cooking by heat inactivates myrosinase, resulting in a 30-60% loss of glucosinolate bioavailability. As a result of the glucosinolate hydrolysis by myrosinase, isothiocyanate and indole derivatives (such as indole-3-carbinol and indole-3-acetonitril) are produced (4). These metabolites from glucosinolate are considered to play a major role in cancer chemoprevention through the induction of detoxification enzymes (5). Many studies have reported that administration of glucosinolate metabolites (isothiocyanate and indol derivatives) and brassica plant extract elevates the phase-I and phase-II enzymes in the rat livers (6, 7).

Bile and pancreatic juice contain a number of parameters for drug metabolism associated with cancer chemoprevention, which include various types of conjugates and detoxifying enzymes. Glutathione-conjugates generate in the liver by glutathione S-transferase and excrete into the gastrointestinal tract through bile. Glutathione-conjugates undergoes to the stepwise cleavage in the small intestine by γ-glutamyl transferase (γ-GTP) that is derived mainly from the pancreas (8, 9). To evaluate the drug metabolism in liver, bile and pancreatic juice should be collected separately because the latter contains high γ-GTP activity (10) that degrades the glutathione-conjugates in bile. Glutathione-conjugates begin to degrade in the bile duct in rats with pancteaticobiliary maljunction. However, there are no reports that analyzes the bile and pancreatic juice separately in rodent models such as mice and rats.

The aim of this study is to develop a method for the selective collection of bile and pancreatic juice in rats. Using this rat model, we evaluated the effects of indole-3-carbinol (I3C) and phenethyl isothiocyanate (PEITC) on excretion rates of bile and pancreatic juice and γ-GTP activity in these samples.

MATERIALS AND METHODS

Chemicals and analytical procedure

The I3C, PEITC, bovine serum albumin, γ-glutamyl-*p*-nitroanilide, glycylglycine and *p*-dimethylaminocinnaldehyde used in this study were purchased from Sigma Chemicals (St Louis, MO, USA). Protein concentration was determined in duplicate by the method of Lowry *et al.* (11) using bovine serum albumin as a standard. γ-GTP activity was measured according to the method described by Igarashi *et al.* (12) employing γ-glutamyl-*p*-nitroanilide as a donor substrate and glycylglycine as a acceptor substrate. The production of *p*-nitroaniline was quantified by measuring the absorbance at 565 nm after chromogenization by the addition of *p*-dimethylaminocinnaldehyde.

Experimental design

The protocol for the animal experiment performed in this study is shown in Figure 1. Male

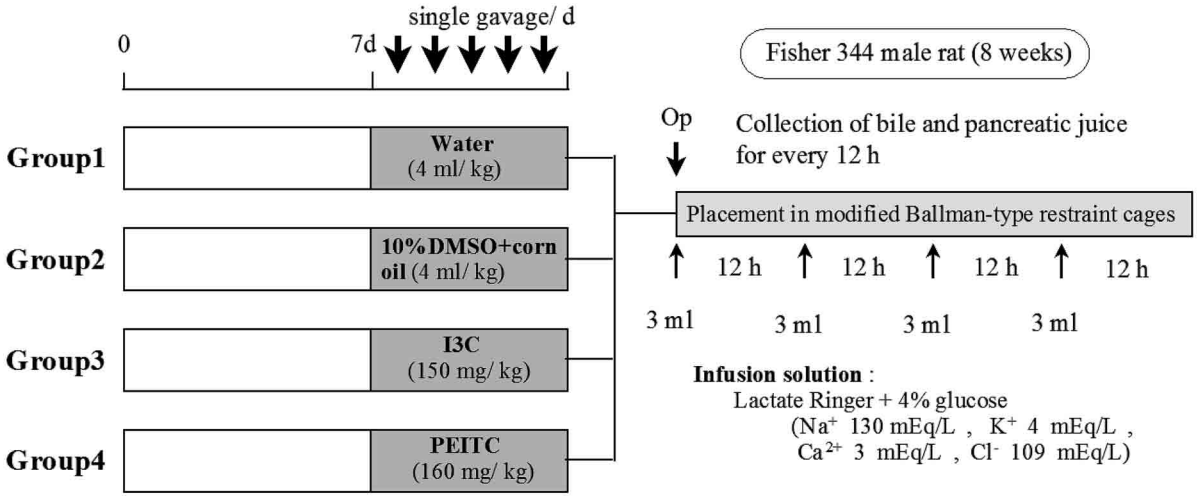

Figure 1. Experimental protocol and design for the bile and pancreatic juice collections. After acclimation for one week, male Fisher344 rats (eight weeks of age) were divided into four groups and intragastrically administered with tap water, 10% DMSO plus corn oil (vehicle), I3C (150 mg/kg), or PEITC (160 mg/kg) for five days. The rats were operated on at 24 h after the final administration as shown in Figure 2, and the bile and pancreatic juice were separately collected for 48 h. The bile and pancreatic juice excretions were measured every 12 h.

Fisher344 rats (eight weeks of age) were purchased from Japan SLC and housed in polycarbonate cages (four rats/cage) containing hard-wood chip bedding. The rats were allowed to access to standard chows and tap water *ad libitum*. After acclimation for one week, the rats were divided into four groups and intragastrically administered with tap water, 10% DMSO plus corn oil (vehicle), I3C (150 mg/kg), or PEITC (160 mg/kg) for five days. The rats were operated on at 24 h after the final administration as described below, and the bile and pancreatic juice were separately collected for 48 h. The bile and pancreatic juice excretions were measured every 12 h. The samples were frozen at -80C until use.

The present study was conducted in compliance with the Division for Animal Research Resources, Institute of Health Biosciences, University of Tokushima. The experiments and procedures were approved by the Animal Care and Use Committee of the University of Tokushima.

Operative procedure

Adult male F344 rats with body weights around 200 g underwent laparotomies under anesthesia, and a 24G infusion needle was inserted into the caudal vein for drop infusion. As shown in Figure 2A, after the duodenum was turned to the left, the common bile duct and pancreatic duct were exposed from the back side of the pancreas. A polyethylene tube with a 0.8-mm diameter was inserted into the tip of a silicon tube with 1-mm diameter to make the 3-mm overhang (Figure 2B). Under observation with a magnifying glass (×3.3), the end of the common bile duct was ligated proximally to the ampulla of Vater, and the cannula shown in Figure 2B was inserted above the ligature to collect pure pancreatic juice. The cannula was fixed by 5-0 silk. Another cannula was inserted into the common bile duct at upstream of the pancreatic duct joint to collect pure bile (Figure 2C). The other end of the inserted tube was passed subcutaneously behind the neck and taken outside the body. Both pure bile and pancreatic juice were collected in syringes surrounded by ice.

After the operation, the rats were placed in modified Bollman-type restraint cages and allowed free access to standard chows and tap water. The room temperature was controlled at 22 to 25°C. The rats

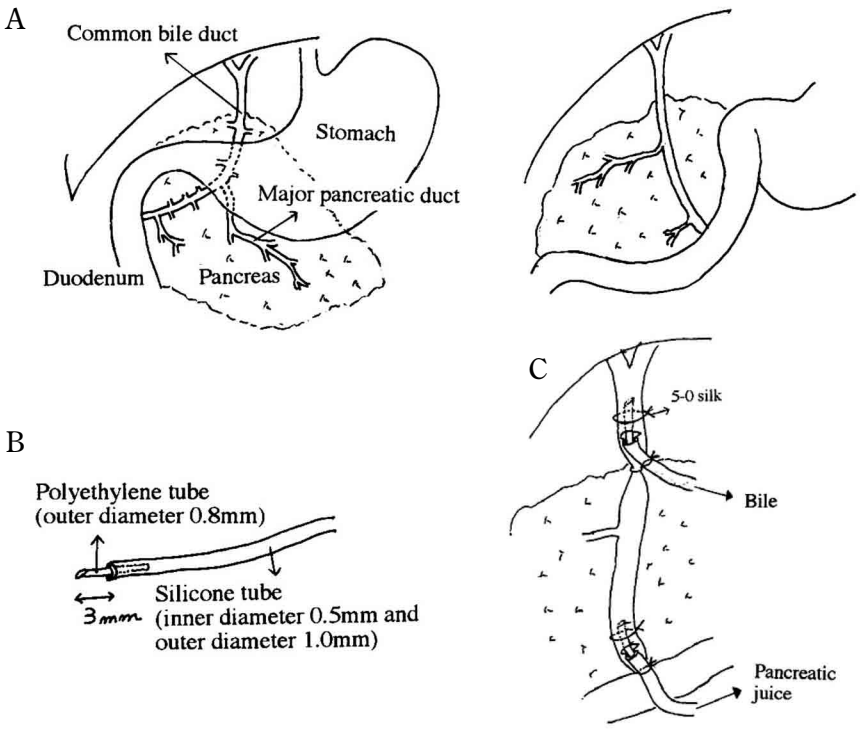

Figure 2. Anatomy of common bile duct and pancreatic duct in rats (A), cannulated tube used in this study (B), and procedure for cannulation (C). Adult male F344 rats with body weights around 200 g underwent laparotomies under anesthesia and after the duodenum was turned to the left, the common bile duct and pancreatic duct were exposed from the back side of the pancreas (panel A). A polyethylene tube with a 0.8-mm diameter was inserted into the tip of a silicon tube with 1-mm diameter to make the 3-mm overhang (panel B). As shown in panel C, the end of the common bile duct was ligated proximally to the ampulla of Vater, and the cannula was inserted above the ligature to collect pure pancreatic juice. The cannula was fixed by 5-0 silk. Another cannula was inserted into the common bile duct at upstream of the pancreatic duct joint to collect pure bile.

received intermittent infusion (Lactate Ringer plus 4% glucose) every 12 h. The injection volume was 3 ml/infusion (for 30 min), and the total infusion volume was 12 ml /48 h.

Statistical analysis

Statistical analysis was performed by one-way ANOVA. All statistical analyses were performed using statistical software (JMP 8.0.1., SAS Campus Drive, Cary, 27513 NC, USA). A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Rat model for collection of bile and pancreatic juice

Cannulation and collection of bile and pancreatic juice were successful in all cases when rats of over 200 g in body weight were used. The excretion volumes in the control rats administered with distilled water were 13.2 ± 1.2 ml/24 h and 21.9 ± 1.4 ml/48 h (34.3 ± 1.7 μl/kg/min) for bile and 4.8 ± 1.7 ml/24 h and 12.8 ± 1.7 ml/48 h (20.4 ± 2.9 μl/kg/min) for pancreatic juice, respectively.

Effects of I3C and PEITC on bile and pancreatic juice excretion

As shown in Table 1, I3C and PEITC administration significantly increased the bile excretion rate for the first 24 h compared with the control rats (corn oil administration). However, bile excretion rate in the rats of these experimental groups tended to decrease for the next 24 h, and no significant difference was observed in the total bile excretion volumes. The total bile excretion for 48 h reached 22.6 ± 2.4 ml (37.2 ± 3.9 μl/kg/min) in the I3C-adnimistered rats and 21.9 ± 1.6 ml (36.7 ± 1.0 μl/kg/min) in the PEITC-administered rats. The protein concentration in bile significantly increased for the first 12 h in the I3C- and PEITC-treated groups but no significant change was observed after 36 h.

The pancreatic juice excretion rate is summarized in Table 2. There was no significant difference in the pancreatic juice excretion rate for the first 36 h between the I3C-treated group and the control group. The total excretion rate significantly decreased when compared with the corn oil-treated group. In the case of PEITC administration, the pancreatic juice excretion rate significantly increased when compared with the corn oil-treated group although no significant difference was found in the total excretion volume. The total pancreatic juice excretion for 48 h reached 14.5 ± 3.2 ml (24.0 ± 5.6 μl/kg/min) in the I3C- administered rats and 20.9 ± 2.6 ml (34.9 ± 2.5 μl/kg/min) in the PEITC-administered rats. The protein concentration in the pancreatic juice significantly decreased for the first 24 h in the PEITC-treated group but no significant

Table 1. Changes of the bile excretion rate

Group No.	Treatment	Bile excretion rate for every 12 h (μl/kg/min)[a]				Total excretion rate (μl/kg/min)[a]
		0-12 h	12-24 h	24-36 h	36-48 h	
1	Water (n=4)	48.8± 5.2	34.2± 0.8	30.5± 4.2	23.6± 2.9	34.3± 1.7
2	Corn oil (n=4)	53.5± 4.3	37.6± 1.9[c]	29.6± 1.8	20.5± 2.6	35.3± 2.0
3	I3C (n=5)	60.2± 9.1[b]	41.7± 1.2[c,e]	27.4± 7.0	19.8± 3.4	37.2± 3.9
4	PEITC (n=6)	58.5± 2.7[b]	41.5± 2.2[c,e]	28.3± 2.6	19.2± 2.4[b]	36.7± 1.0

[a] Mean± S.D.
[b, c] Significantly different from group 1 (b : $p< 0.05$, c : $p< 0.01$)
[d, e] Significantly different from group 2 (d : $p< 0.05$, e : $p< 0.01$)

Table 2. Changes of the pancreatic juice excretion rate

Group No.	Treatment	Pancreatic excretion rate for every 12 h (μl/kg/min)[a]				Total excretion rate (μl/kg/min)[a]
		0-12 h	12-24 h	24-36 h	36-48 h	
1	Water (n=4)	12.3± 6.2	18.2± 4.0	21.8± 6.8	29.4± 8.7	20.4± 2.9
2	Corn oil (n=4)	15.7± 5.0	31.0± 2.9[b]	39.6± 3.9[b]	34.1± 7.4	30.1± 0.8[b]
3	I3C (n=5)	15.7± 7.3	29.4± 9.2[b]	29.6± 8.2	21.6± 6.1[e]	24.0± 5.6[e]
4	PEITC (n=6)	28.8± 1.6[b,d]	42.4± 2.8[b,d]	42.5± 7.7[b]	27.5± 5.5	34.9± 2.5[b]

[a] Mean± S.D.
[b, c] Significantly different from group 1 (b : $p< 0.01$, c : $p< 0.05$)
[d, e] Significantly different from group 2 (d : $p< 0.01$, e : $p< 0.05$)

change was observed after 24 h.

γ-GTP activities in bile and pancreatic juice

γ-GTP activity in pancreatic juice was about 100 times higher than that in bile when the activities were compared in the control group (water administration). As shown in Figure 3, γ-GTP activities in the bile collector from the I3C- and PEITC-treated groups were significantly higher than those in the control group for the first 24 h, but no significant difference was observed after 24 h. On the other hand, as shown in Figure 4, γ-GTP activities in the pancreatic juice of PEITC-treated groups were significantly lower than those in the control group during the 12-24 h period. However, both I3C- and PEITC-treatment provided little difference to γ-GTP activity in pancreatic juice.

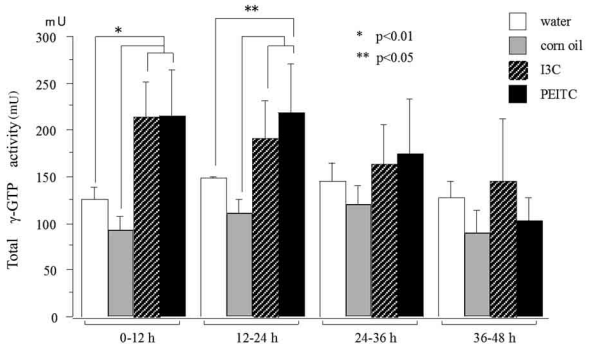

Figure 3. γ-GTP activities in pure bile. γ-GTP activities in the bile collected from I3C- and PEITC-treated groups were significantly higher than those in control group for the first 24 h, but no significant difference was observed after 24 h.

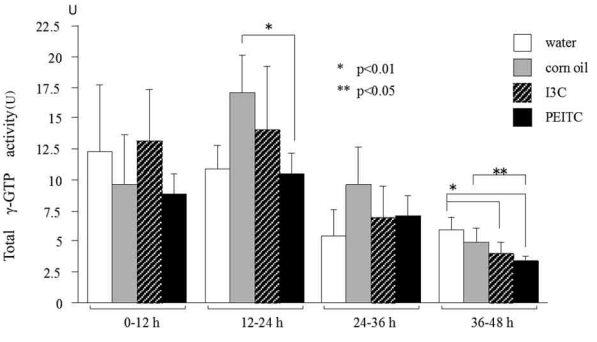

Figure 4. γ-GTP activities in pure pancreatic juice. γ-GTP activities in pancreatic juice of PEITC-treated groups were significantly lower than those in control group during the 12-24 h period. However, both I3C- and PEITC-treatment provided little difference to γ-GTP activity in pancreatic juice.

DISCCUSION

Methods for the collection of both bile (13) and pancreatic juice in a pancreatitis model (14) have been reported, respectively. However, no reports are available for the selective and simultaneous collection of bile and pancreatic juice. In the method described by Toriumi *et al.*, rats with tethered pancreatic fistula were partially restrained by tube (14). We have tried this model but the rats sometimes bit and removed the catheter. To solve this problem, we employed Bollman cages for their short-term restraint. Since restraint using Bollman cage results in dehydration due to insufficient water intake, which leads to the reduction of bile and pancreatic juice excretion, infusions with Lactate Ringer were performed to keep the bile and pancreatic juice excretion stable. For the cannulation into the bile or pancreatic duct, we constructed a modified cannulation tube that comprised of a hard tip for cannulation into the bile or pancreatic duct and a soft tube for drainage to an extra body (Figure 2B). This cannula made it easy to stably collect the bile and pancreatic juice, and we encountered no cannulation-associated complications. Because the bile and pancreatic ducts of rats are thin (around 1 mm in diameter), training is necessary for cannulation. However, once established, the short-term collection of bile and pancreatic juice can be stably performed.

Prevention of cancer initiation can be achieved by limiting the exposure of cells to carcinogenic substances by either by inhibiting their activation or increasing their detoxification and subsequent removal (15). Moreover, cancer chemopreventive compounds can suppress promotion and progression of carcinogenesis by interfering with various signaling pathways involving oxidative stress (16), inflammation (17), and cellular proliferation. Additionally, some of these compounds can show their cancer chemopriventive effects at all of the stages of carcinogenesis (initiation, promotion, and progression) by inducing the cell cycle arrest and apoptosis (18, 19).

Epidemiological studies have shown that consumption of brassica plants during adolescence was associated with a 72% reduction of the risk of breast cancer (20). Cabbage consumption has also been reported to be associated with a reduction of the risks of developing prostate and pancreatic cancers (21, 22). The anti-cancer effect of brassica plants derives partly from the glucosinolate and its hydrolytic metabolites such as I3C and PEITC (ref 4).

These anti-cancer agents are considered to induce detoxification enzymes (5-7), of which the Phase II enzymes such as glutathione-S-transferase especially play an especially important role (23, 24). As a result of Phase II reaction, the glucuronate-, sulfate- and glutathione-conjugates are produced and excreted into bile or urine. It has been reported that high γ-GTP activity is present in the pancreas and in pancreatic juice (8-10), and glutathione-conjugates are converted into cysteinylglycine-conjugates by this enzyme activity in pancreatic juice. Cysteinylglycine-conjugates are further metabolized to cysteine-conjugates by aminopeptidase in pancreatic juice (phase III reaction). Thus, γ-GTP is one of the key enzymes for detoxification, which degrades γ-glutamyl compounds and catalyzes the first reaction of the metabolism of glutathione conjugates to mercapturic acid in Phase II reaction.

In this study, we investigated the effect of the anti-cancer agents, I3C and PEITC, on the excretion of bile and pancreatic juice. Bile excretion was significantly increased for the first 24 h in both the I3C- and PEITC-treated groups. In addition, γ-GTP activities were also elevated in these groups. These results indicate that I3C and PEITC enhance bile excretion and billiary γ-GTP activity. Furthermore, the excretion of pancreatic juice was also increased for the first 24 h in PEITC-treated rats although the γ-GTP activity in pancreatic juice was decreased by PEITC-treatment, possibly leading to the suppression of Phase II reaction. I3C and PEITC might enhance the detoxification and excretion of the metabolites of mutagenic compounds by increasing bile and pancreatic juice excretion and changing the γ-GTP activity in bile and pancreatic juice.

In summary, our rat model for the selective and simultaneous collection of bile and pancreatic juice is expected to work as a useful tool for the analyses of drug metabolism and anti-cancer agents like I3C and PEITC.

CONFLICT OF INTEREST

The authors have no conflict of interest associated with the present study.

REFERENCES

1. McNaughton SA, Marks GC : Development of a food composition database for the estimation of dietary intakes of glucosinolates, the biologically active constituents of cruciferous vegetables. Br J Nutr 90 : 687-697, 2003
2. Higdon JV, Delage B, Williams DE, Dashwood RH : Cruciferous vegetables and human cancer risk : epidemiologic evidence and mechanistic basis. Pharmacol Res 55 : 224-236, 2007
3. Verhoeven DT, Verhagen H, Goldbohm RA, van den Brandt PA, van Poppel G : A review of mechanisms underlying anticarcinogenecity by brassica vegetables. Chem Biol Interact 103 : 79-129, 1997
4. Holst B, Williamson G : A critical review of the bioavailability of glucosinolates and related compounds. Nat Prod Rep 21 : 425-447, 2004
5. Staack R, Kingston S, Wallig MA, Jeffery EH : 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
6. Szaefer H, Krajka-Kuźniak V, Bartoszek A, Baer-Dubowska W : Modulation of Carcinogen Metabolizing Cytochromes P450 in Rat Liver and Kidney by Cabbage and Sauerkraut Juices : Comparison with the Effects of Indole-3-carbinol and Phenethyl Isothiocyanate. Phytother Res. 2011 Dec 15. [Epub ahead of print]
7. Krajka-Kuźniak V, Szaefer H, Bartoszek A, Baer-Dubowska W : Modulation of rat hepatic and kidney phase II enzymes by cabbage juices : comparison with the effects of indole-3-carbinol and phenethyl isothiocyanate. Br J Nutr 105 : 816-826, 2011
8. Kinouchi T, Kataoka K, Miyanishi K, Akimoto S, Ohnishi Y : Biological activities of the intestinal microflora in mice treated with antibiotics or untreated and the effect of the microflora on absorption and metabolic activation of orally administered glutathione conjugates of K-region epoxides of 1-nitropyrene. Carcinogenesis 14 : 869-874, 1993
9. Kinouchi T, Nishifuji K, Ohnishi Y : Biliary excretion of glutathione conjugates of 4,5-epoxy-4,5-dihydro-1-nitropyrene and 9,10-epoxy-9,10-dihydro-1-nitropyrene in rats administered 1-nitropyrene orally and their further metabolism in the intestinal tract. Carcinogenesis 11 : 1381-1387, 1990
10. Battistini B, Chailler P, Brière N, Beaudoin AR. Secretion of gamma-glutamyltranspeptidase by

the pancreas : evidence for a membrane shedding process during exocytosis. Life Sci. 47 : 2435-2441, 1990
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ : Protein measurement with the Folin phenol reagent. J Biol Chem 193 : 265-275, 1951
12. Igarashi T, Satoh T, Ueno K, Kitagawa H : Species difference in glutathione level and glutathione related enzyme activities in rats, mice, guinea pigs and hamsters. J Pharmacobiodyn 6 : 941-949, 1983
13. Tomlinson PW, Jeffery DJ, Filer CW : A novel technique for assessment of biliary secretion and enterohepatic circulation in the unrestrained conscious rat. Xenobiotica 11 : 863-870, 1981
14. Toriumi Y, Samuel I, Wilcockson DP, Joehl RJ : A new model for study of pancreatic exocrine secretion : the tethered pancreatic fistula rat. Lab Anim Sci 44 : 270-273, 1994
15. Hanausek M, Walaszek Z, Slaga TJ : Detoxifying cancer causing agents to prevent cancer. Integr Cancer Ther 2 : 139-144, 2003
16. Klaunig JE, Kamendulis LM : The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 44 : 239-267, 2004
17. Karin M : Nuclear factor-kappaB in cancer development and progression. Nature 441 : 431-436, 2006
18. Cheung KL, Kong AN : Molecular targets of dietary phenethyl isothiocyanate and sulforaphane for cancer chemoprevention. AAPS J 12 : 87-97, 2010
19. Aggarwal BB, Ichikawa H : Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. Cell Cycle 4 : 1201-1215, 2005
20. Nelson NJ : Migrant studies aid the search for factors linked to breast cancer risk. J Natl Cancer Inst 98 : 436-438, 2006
21. Kristal AR, Lampe JW : Brassica vegetables and prostate cancer risk : a review of the epidemiological evidence. Nutr Cancer 42 : 1-9, 2002
22. Larsson SC, Håkansson N, Näslund I, Bergkvist L, Wolk A : Fruit and vegetable consumption in relation to pancreatic cancer : a prospective study. Cancer Epidemiol Biomarkers Prev 15 : 301-305, 2006
23. Nijhoff WA, Grubben MJ, Nagengast FM, Jansen JB, Verhagen H, van Poppel G, Peters WH : Effects of consumption of Brussels sprouts on intestinal and lymphocytic glutathione S-transferases in humans. Carcinogenesis 16 : 2125-2128, 1995
24. Staack R, Kingston S, Wallig MA, Jeffery EH : 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