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CHEMOPREVENTION OF COLON CANCER BY THE KOREAN FOOD STUFFS COMPONENTS

Dae Joong Kim*, Dong Hwan Shin, Byeongwoo Ahn, Dong Deuk Jang, Hiroyuki Tsuda[1], and Shoji Fukushima[2]

National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, Korea, [1]Experimental Pathology and Chemotherapy Division, National Cancer Center Research Institute, Tokyo, Japan, and [2]First Department of Pathology, Osaka University Medical School, Osaka, Japan

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Dae Joong Kim*, Dong Hwan Shin, Byeongwoo Ahn, Dong Deuk Jang, Hiroyuki Tsuda[1], and Shoji Fukushima[2]

Cancer Prevention Laboratory, Department of Pathology, National Institute of Toxicology Research, Korea Food and Drug Administration, Nokbun-dong, Eunpyung-ku, Seoul 122-704, Korea; [1]Experimental Pathology & Chemotherapy Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chou-ku, Tokyo 104-0045; [2]First Department of Pathology, Osaka City University Medical School, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan .

Running Title: Chemoprevention of Colon Cancer by I3C

*To whom correspondence should be addressed.

Corresponding Address:
Dr. Dae Joong Kim, DVM, PhD,
Department of Pathology, National Institute of Toxicology Research,
Korea Food and Drug Administration,
Nokbun-dong, Eunpyung-ku,
Seoul 122-704, Korea.

Tel: +82-2 380-1822; Fax: +82-2 380-1820
E-mail: dkim@kfda.go.kr

Abbreviations: Familial adenomatous polyposis (FAP); *APC* (*adenomatous polyposis coli*) gene; Inducible cyclooxygenase (COX-2); Non-steroidal anti-inflammatory drugs (NSAIDs); Inducible nitric oxide synthase (iNOS, NOS-2); indole-3-carbinol (I3C); C57BL/6J-*Apc*$^{Min/+}$ mice (*Min*/+ mice).

ABSTRACT

Inducible cyclooxygenase (COX-2) and inducible nitric oxide synthase (iNOS, NOS-2) play pivotal roles as mediators of inflammation involved in early steps of carcinogenesis in certain organs. Therefore, chemoprevention is conceivable with inhibition of COX-2 and/or iNOS. In the present study, we examined the chemopreventive effects of indole-3-carbinol (I3C), a constituent of cruciferous vegetables (the Family of *Cruciferae*) such as cabbages, cauliflowers and broccoli on multiple intestinal neoplasia (Min) genetic mouse model and on mouse colon carcinogenesis induced by azoxymethane (AOM).

Powdered AIN-76A diets (Harlan Teklad Research Diets, Madison, USA) containing 100 or 300 ppm I3C (groups 1 or 2) or the same diet pelleted without supplement (group 3) were fed to 6 week old male C57BL/6J-*Apc*$^{Min/+}$ (*Min*/+) mice (The Jackson Laboratory, Bar Harbor, ME, USA) for 10 weeks. In additional the same diets were given to wild-type normal C57BL/6J-*Apc*$^{Min/+}$ littermates after AOM initiation (groups 4 – 7: 10 mice in each group) for 32 weeks from week 4. At 16 weeks of age, all *Min*/+ mice (groups 1-3) were sacrificed for assessment of intestinal polyp development. The incidences of the colonic adenomatous polyps in the group 1, 2 and 3 were 60% (12/20), 60% (15/25) and 84% (21/25), respectively. A decreasing tendency in multiplicities of the colonic adenomatous polyps in the group 1 (I3C 100 ppm; 0.85±0.22; 61%) and the group 2 (I3C 300 ppm; 1.32±0.28; 94%) was observed when compared with group 3 (control; 1.40 ±0.21; 100%). Total numbers of aberrant crypt foci (ACF) or aberrant crypts (AC)/colon in wild type mice of groups 4 or 5 were decreased significantly compared with those of the AOM alone group (group 6) ($P<0.01$). These results suggest that I3C may be a potential chemopreventive agent for colon cancer.

Key words : Chemoprevention; Colon cancer; Familial adenomatous polyposis (FAP); *APC* (*adenomatous polyposis coli*) gene; Multiple intestinal neoplasia (Min); Azoxymethane (AOM); Inducible cyclooxygenase (COX-2); Inducible nitric oxide synthase (iNOS, NOS-2); Indole-3-carbinol (I3C); C57BL/6J-*Apc*$^{Min/+}$ mice (*Min*/+ mice); Aberrant crypt foci (ACF); Aberrant crypts (AC).

INTRODUCTION

Chemoprevention, on the basis that carcinogenesis is multistep molecular and cellular process, may be an effective way to reduce cancer risk. For this purpose, noncytotoxic nutrients or pharmacological compounds that protect against the development and progression of mutant clones into malignant cells was be employed (1-3). There has been a great deal of interest in chemoprevention of colorectal, breast, and prostate cancer during the past two decades in the USA (4). Rapid advances in two important areas, the development of a genetic model for colorectal tumorigenesis and human chemoprevention research, have brought about new possibilities for innovative approaches to the prevention and control of colorectal cancers in particular.

Vogelstein *et al.* (5), Fearon and Vogelstein (6), and Fearon and Jones (7) have developed a preliminary genetic model for colorectal tumorigenesis, a series of genetic mutations being thought required for malignant tumor development, with the order of occurrence of the genetic mutations being less important than their number. Molecular events that cause such genetic mutations are thus possible targets for chemoprevention of colorectal cancer progression (3,5-7).

Molecular genetic studies of familial adenomatous polyposis (FAP) families led to the discovery of the tumor suppressor gene *APC* (*adenomatous polyposis coli*) located on human chromosome 5q21 (8,9). Mutations in *APC* appear to be responsible not only for FAP but also many sporadic cancers of the colorectal axis, stomach, and esophagus (9). The pathogenesis of colorectal cancer has been characterized as a multistep process that begins with increased proliferation and/or decreased apoptosis of epithelial cells, followed by adenoma formation and dysplasia, invasion, and ultimately, metastasis (9). Alterations of tumor suppressor genes and oncogenes have been identified at various stages of tumorigenesis. *APC* gene mutations predispose to adenoma formation, an early event in the multistep process of the colon tumorigenesis (9,10).

Epidemiological studies have shown that prolonged use of aspirin is associated with reduced risk of colorectal cancer (11) and several non-steroidal anti-inflammatory drugs (NSAIDs) have been found to suppress development of chemically induced colon carcinomas in rats and intestinal polyps in multiple intestinal neoplasia (Min) mice (12-14). Although the mechanisms have yet to be clarified, the drug has shown to induce apoptosis, possibly by inhibiting COX-2 (13,14). Several investigators have previously reported inducible cyclooxygenase (COX-2) expression to be increased in human colorectal adenocarcinomas when compared with normal adjacent colonic mucosa (15-17). Markedly elevated levels of COX-2 mRNA and protein are expressed in colonic tumors of rodents induced by azoxymethane (AOM, a colon carcinogen) (18,19) and in adenomas taken from Min mice, which have germline nonsense mutation at colon 850 of *Apc* gene and spontaneously develop multiple polyps in their small and large intestines (20). COX-2 mRNA and protein were also recently found to be overexpressed in gastric carcinomas (21), squamous cell carcinomas (22,23), and lung carcinomas (24). Koga *et al.* demonstrated a high expression of COX-2 in well-differentiated hepatocellular carcinoma (HCC) and a low expression in advanced HCC in human suggesting roles for this enzyme in cellular differentiation of hepatic tumors (25). Oshima *et al.* have provided evidence that COX-2 may play an extremely important role in the development of adenomas following loss of *APC* function (26). The heterozygous C57BL/6J-*Apc*$^{Min/+}$ mice (*Min*/+; The Jackson Laboratory, Bar Harber, ME, USA) develop numerous intestinal polyps, and can serve as a murine model of human FAP (13,14). Current strategies for colorectal cancer control include dietary modification, removal of adenomatous polyps by surgery, colectomy, or use of NSAIDs (14,27).

Some of the best characterized candidate chemopreventive agents to date are NSAIDs, which have been shown to be effective against the development of several types of solid tumors, particularly in urinary bladder and intestine (28). NSAIDs are a chemically diverse family of agents which share the ability to inhibit the activity of cyclooxygenases, a key enzyme in the metabolic conversion of

arachidonic acid to a variety of bioactive lipids including prostaglandins, thromboxanes and leukotrienes (29). Several independent lines of experimental evidence now suggest that NSAIDs can be effective inhibitors of intestinal tumor formation, and that they exert the inhibitory role of cyclooxygenase function and the induction of apoptosis (30).

The use of chemopreventive substances or mixtures of substances from natural sources is a desirable approach that appears to be particularly appropriate for colorectal tumorigenesis (2,3). Explorative research with indole-3-carbinol (I3C) has demonstrated it to be a potential chemopreventive agent for the colon, mammary gland, stomach, and liver cancers in animals as well as for human cell lines (31-35). Among various indoles, I3C has received particular interest as a possible cancer chemopreventive agent and this is reflected in the number of citations in the medical literature (36,37). However, evidence of promotion or enhancement of carcinogenesis by I3C has also been obtained (31,32,36,38). In the present study, we examined the modification effects of I3C, a constituent of cruciferous vegetables such as cabbages, cauliflowers and broccoli on Min mouse intestinal tumorigenesis and on mouse colon carcinogenesis induced by AOM.

MATERIALS AND METHODS

Animals and chemicals

Seventy 6-week-old male heterozygous C57BL/6J-*Apc*$^{Min/+}$ (*Min*/+) mice and forty normal wild-type C57BL/6J-*Apc*$^{Min+/+}$ littermates (*Min*+/+) were supplied by The Jackson Laboratory, Bar Harbor, ME, USA. The animals were kept in the Laboratory Animal Care Facility of the National Institute of Toxicology Research (Seoul, Korea) in a room with a 12 h light-dark cycle and controlled humidity and temperature (23±2 °C, 55±10% RH), in polycarbonated cages with absorbent hardwood bedding (Beta-chips, Northeastern Products Co., Warrensbury, NY, USA). They were allowed free access to tap water and pelleted chow (AIN-76A, Harlan Teklad Research Diet, Madison, Wisconsin, USA). AIN-76A pellet or powdered diets were obtained from the Harlan Teklad Research Diet, Madison, Wisconsin, USA (Table 1). Indole-3-carbinol (I3C; CAS No. 700-06-1, I-7256) and azoxymethane (AOM, CAS 25843-45-2, A-9517) were purchased from the Sigma Chemical Co., St. Louis, MO, USA (Figure 1).

Treatments

Seventy 6-week-old male heterozygous C57BL/6J-*Apc*$^{Min/+}$ (*Min*/+) mice were randomly divided into three groups (Experimental protocol: Figure 2). Animals of groups 1-3 (20 or 25 mice) (Figure 2) were placed on AIN-76A pellet diet (Harlan Teklad, Madison, Wisconsin, USA) (group 3) or the same powdered diet containing 100 or 300 ppm I3C (groups 1 or 2) for 10 weeks. As an additional experiment using AOM (colon carcinogen), forty male C57BL/6J-*Apc*$^{Min+/+}$ littermates (*Min*+/+) mice were randomly divided into four subgroups (groups 4-7) (Experimental protocol: Figure 3). The animals were subcutaneously injected with AOM (5 mg/kg body weight, four times at weekly interval) or saline as a vehicle for induction of colon tumors (39,40). Then wild type mice

were placed on the AIN-76A pellet (group 6) or AIN-76A powdered diet (groups 4,5,7) containing of I3C (100 or 300 ppm) for 32 weeks (Experimental protocol: Figure 3). At 17 weeks of age all Min/+ mice in groups 1-3 were sacrificed to examine intestinal polyp development. Mice of groups 4-7 were sacrificed for examination of aberrant crypts (AC) or aberrant crypt foci (ACF) in the colonic mucosa and to process the intestines for histopathological findings at 42 weeks of age (41-44).

Polyp number scoring

At the scheduled age, polyps were counted according to the method described previously except that the gut was inflated with 10% formaldehyde in PBS facilitated, before being opened longitudinally (24). The procedure made the gut epithelium well distended, and facilitated counting and sizing of the nascent uni-villous polyps. All of the polyps (from the duodenum to rectum) were measured with an IBAS automatic image analysis system (Kontron Co., Ltd., Germany). The total number of tumors in each section of the small intestine (three equal parts) and in the large intestines was scored by trans-illumination with an inverted light microscope.

Histological examination

Sections of small intestine and large intestine mucosa were also examined. Specimens 5mm in thickness were fixed in 10% neutral phosphate buffered formalin, embedded in paraffin, sectioned at 3μm, and stained with hematoxylin and eosin (H&E) for the evaluation of mucosal histology after polyp and ACF counting (41-44). For measuring ACF and AC of the colon (groups 4-7), colon samples were stained with methylene blue (41-44).

Statistical analysis

Statistical analyses were performed with the SAS (Statistical Analysis System) software. Tumor

incidences in the small and large intestines were compared with the χ^2 test and tumor multiplicities with the Kruskal-Wallis test. The significance of differences in diagnosis was tested by the redit test. All of the test were followed by the Duncan's multiple range test as a post hoc test was applied. For all comparisons, probability values less than 5% ($P<0.05$) were considered to be statistically significant.

RESULTS

Polyps in the small intestines

Tumor multiplicity data for the small intestines are summarized in Table 2. The total numbers of polyps did not significantly differ among the groups, although the I3C low dose group (group 1) showed a tendency for decrease in proximal part (the upper one-thirds of the whole small intestines), when compared with control group (AIN-76A diet) (Table 2).

Polyps in the large intestines

The incidences of the colonic adenomatous polyps in groups 1, 2, and 3 were 60% (12/20), 60% (15/25) and 84% (21/25), respectively. The multiplicities of colonic adenomatous polyps in groups 1, 2, and 3 were 0.85±0.22 (I3C 100 ppm), 1.32±0.28 (I3C 300 ppm), and 1.40±0.21 (control), respectively with no significant variation (Table 3).

AC and ACF of the colonic mucosa

Data for ACF in wild type littermates mice are shown in Table 4. The total numbers of ACF/colon and AC/colon in the I3C groups were significantly decreased as compared to the control values (group 7). As expected, wild type normal littermates of group 7 receiving I3C alone had no lesions in the small or large intestines at week 42 of age. The mean sizes of ACF in groups 1, 2, and 3 were 1.8±0.71 (78%), 2.1±0.38 (91%), and 2.3±0.38 (100%), respectively (Table 4).

DISCUSSION

Our results suggest that I3C may have the potential chemopreventive effects on the development of intestinal tumors in a murine model of human FAP as well as on development of preneoplastic lesions in a colon carcinogenesis model induced by AOM. The fact that formation of polyps smaller than 3 mm in diameter was reduced in the former case suggests that more substantial results would be obtained with younger *Min*/+ mice (3 or 4-weeks-old). Clarification of whether I3C actually brings about a reduction in COX-2 or prostaglandin-related pathways in mouse colorectal lesions is also necessary.

Studies of human colorectal cancers have revealed COX-2 to be increased in about 90% of cases. It is also expressed in 40% of premalignant colorectal adenomas, but not in nontumor colonic mucosa (17). NSAIDs, agents that block the activity of COX-2, are associated with a decreased incidence of colon cancer in humans (16,17). In patients with FAP, the NSAID sulindac is also effective at mediating regression of colorectal adenomas (45). Treatment with NSAIDs is associated with a decrease in COX-2 in colonic tumors (13,16). There is now considerable evidence, from several different experimental systems, that COX-2 may play a pivotal role in the genesis of colorectal cancer (26,44,46).

NSAIDs have been shown to suppress the development of chemically induced colon carcinomas in rats and intestinal polyps in *Min*/+ mice (12,14). Although the mechanisms by which NSAIDs causes regression of adenomas have yet to be clarified, they may induce apoptosis, possibly by their effects on COX-2 (13, 14). Overexpression of COX-2 has been observed in established *Min*/+ mice tumors (13). Although most intestinal tumor initiation is thought to occur within the first month of life in Apc^{Min} mice (47), it has been reported that new tumors continue to appear throughout the lifespan of B6-Apc^{Min} mice (48). COX-2 and/or iNOS (NOS-2) play pivotal roles as mediators of

inflammation involved in early step of certain types of carcinogenesis (49). Our recent studies also demonstrate a marked inhibition of tumor growth by treatment with 5,5'-1,4-phenylene-bis(1,2-ethanediyl)bis-isothiourea, a highly selective iNOS inhibitor in *Apc*^{Min} mice (data not shown) as well as in rat colon carcinogenesis induced by AOM (49). A major question that remains to be answered is which signaling pathways are involved downstream of the COX-2 or COX-3 enzyme? These could provide additional molecular targets for cancer prevention studies in the near future (50).

Naturally occurring components have received considerable attention as potential chemopreventive agents. Colorectal cancer is a main cause of cancer death in Western countries, for example accounting for 15% of all cancer patients and over 60,000 mortalities annually in the USA (4). Although the etiology of colon cancer is considered to be multifactorial and complex, dietary factors like a high animal fat intake are considered to be positively linked with an elevated incidence. The mortalities caused by colorectal cancer in the Korean populations are very much less than in Western countries (2), presumably related to consumption of larger amounts of cruciferous vegetables such as Korean cabbage, cabbage, radish, and others. We believe and convince that naturally occurring food components which having COX-2 and/or iNOS inhibitors provide advantages over the NSAIDs as probably safer and more effective chemopreventive agents against colon cancer in our life. Our working hypothesis is that our own traditional life styles of the Korean population may have a bearing on chemoprevention elsewhere with special importance for dietary habitual customs.

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Table 1. The composition of the AIN-76A purified diet

Ingredient components	g/kg
Casein, High protein	200.0
DL-Methionine	3.0
Sucrose	499.99
Corn starch	150.0
Corn oil	50.0
Fiber (cellulose)	50.0
Mineral mix., AIN-76 (170915)	35.0
Vitamin mix., AIN-76A (40077)	10.0
Choline Bitartrate	2.0
Ethoxyquin (antioxidant)	0.01

AIN-76A diet was purphased from Harlan Teklad Research Diet, Madison, Wisconsin, USA.

Table 2. Multiplicities of small intestinal polyps in C57BL/6J-*Apc*$^{Min/+}$ mice treated with I3C

Group Treatment	No. of mice	Multiplicities of intestinal polyps			
		Duodenum	Jejunum	Ileum	Total
1. Min/+ → I3C 100	20	3.11±0.85	9.42±1.81	19.58±4.25	32.11±5.97
2. Min/+ → I3C 300	25	1.56±0.47	9.24±1.31	22.68±2.78	33.48±3.63
3. Min/+	25	1.48±0.35	11.0±1.46	22.52±3.56	35.04±4.89

Data are Mean ±SE values.

The small intestinal polyps were observed in all mice in the Min/+.

Min/+ mice were given AIN-76A diet containing I3C (100 or 300 ppm).

Table 3. Incidences and multiplicities of colonic polyps in C57BL/6J-*Apc*$^{Min/+}$ mice treated with I3C

Group Treatment	No. of mice	Total polyps			Incidence (%)	Multiplicity[b] (%)[c]
		≤3.0mm[a]	>3.0mm	Sum		
1. Min/+ → I3C 100	20	6	11	17	12/20 (60)	0.85±0.23 (61)[c]
2. Min/+ → I3C 300	25	23	10	33	15/25 (60)	1.32±0.29 (94)
3. Min/+	25	25	10	35	21/25 (84)	1.40±0.24 (100)

a: Polyp diameter (mm).

b: Data (Mean± SE) are total numbers of colonic polyps/mouse.

c: The values represent the ratios relative to the control group.

Min/+ mice were given AIN-76A diet containing I3C (100 or 300 ppm).

Table 4. Data for ACF and AC in C57BC/6J mice given AOM and/or I3C at 36 weeks

Group Treatment	No. of mice	No. of ACF		Total No. ACF/colon	Total No. AC/colon	Mean size (AC/ACF)
		∑≤3AC	∑≥4 AC			
4. AOM → I3C 100	10	9.6±5.03** (46)[a]	0.6±0.88 (27)	10.1±5.09** (44)	20.1±10.13** (38)	1.8±0.71 (78)
5. AOM → I3C 300	10	9.2±5.24** (44)	1.2±1.39 (55)	10.4±5.61** (43)	21.2±11.30** (40)	2.1±0.38 (91)
6. AOM→AIN-76A	10	20.8±36.0 (100)	2.2±2.39 (100)	23.0±6.89 (100)	52.6±21.59 (100)	2.3±0.33 (100)
7. I3C 300 alone	10	0	0	0	0	0

Data are Mean ± SD values.

ACF: Aberrant crypt foci. AC: Aberrant crypts.

AOM: Azoxymethane (5mg/kg body weight, four times s.c. injection at weekly intervals).

a: The values are ratios relative to the control group.

All wild type C57BL/6J mice were injected with AOM or saline for colon carcinogenesis.

I3C: Diets containing 100 or 300 ppm of I3C given for 32 weeks.

**: Significantly different from the AOM alone group values at $P<0.01$.

Abstract in Korean

한국전통식품의 대장암 예방 효과

김대중*, 신동환, 안병우, 장동덕, Hiroyuki Tsuda, Shoji Fukushima
국립독성연구소 병리부

형질전환 (유전자 결핍; knockout) Min 마우스를 이용하여 대장암 발생에서 배추, 양배추 주성분인 indole-3-carbinol (I3C)의 대장암 예방효과를 규명하고자 하였다. 실험동물로는 C57BL/6J-*Apc*$^{Min/+}$ (Min 마우스)계의 5 내지 6 주령의 수컷 이형접합체 형질전환 마우스 70 마리와 C57BL/6J 계의 동일 산자, 동일 주령의 수컷 wildtype 비형질전환 마우스 10 마리를 The Jackson Laboratory 사 (Bar harber, ME, USA)로부터 직접 구입하였다. C57BL/6J- *Apc*$^{Min/+}$계 수컷 이형접합체 형질전환 (Min)마우스 70 마리를 각 군 20 내지 25 마리씩 세군으로 나누었다. Group 1 에는 20 마리, Group 2 에는 25 마리, Group 3 에는 25 마리를 배치하고, I3C 투여 실험군 (Group 1 과 2)에는 실험시작시에 AIN-76A 분말사료에 I3C 가 각각 100 및 300ppm 이 함유되도록 조제하여 공급하였다. 그리고 실험군 (Group 3)에는 실험시작부터 종료시까지 AIN-76A 정제고형사료(Teklad 사, WI, USA)를 자유로이 급이하였다. 각 군간의 체중, 사료 및 음수소비량을 매 2 주마다 측정하였고, 10 주간 (16 주령)의 실험종료시에는 최종체중과 간장, 신장, 비장, 등의 장기무게를 측정하여 상대장기 무게비를 산출하였다. 대조군으로서 C57BL/6J 계의 동일 산자, 동일 주령의 수컷 wildtype 비형질전환 마우스 10 마리는 같은 조건의 사육실에서 AIN-76A 정제고형사료를 33 주간 자유로이 급이하였다. 실험동물은 부검전에 하룻밤 동안 절식하고 이산화탄소 흡입 마취하에서 흉대동맥을 절단하여 방혈하고 각 장기 (심장, 폐, 위)를 적출하여 생리식염수에 넣어 장기무게를 측정하고 포르말린에 고정하였다. 소장과 대장의 검사를 위하여 위의 식도부위와 직장을 실로 결찰하여 적출하고 생리식염수를 주입하여 팽창시켜, 십이지장, 공장, 및 회장, 그리고 대장으로 나누어 여과지에 펼친 후 포르말린에 고정하였다. 소장과 대장은 육안 및 자동영상분석기를 이용한 분석이 끝난 후에 각 부위별로 4-6 개의 절편을 작제하여 포르말린에 재고정하고, 통상적인 조직처리과정, 파라핀 포매 및 3-4 μm 두께의 조직절편을 제작하여 H&E 염색을 실시하여 현미경으로 검경하였다. 약 1 주일간의 포르말린 고정이 끝난 소장 및 대장을 부위별, 크기별 종양개수 및 분포를 자동영상분석기 (Kontron Co. Ltd., Germany)로 분석하였다. 체중의 변화, 장기무게, 사료소비량, 및 마리당 종양의 개수에 대한 통계학적 유의성 검증을 위하여 Duncan's t-test 로 통계처리 하였고, 종양 발생빈도에 대하여는 Likelihood ratio Chi-square test 로 유의성을 검증하였다. C57BL/6J-*Apc*$^{Min/+}$계 수컷 이형접합체 형질전환 마우스에 AIN-76A 정제사료만을 투여한 대조군의 대장선종의 발생률은 84% (Group 3; 21/25 례)로써 I3C 100ppm 및 300ppm 을 투여한 경우에 있어서는 각군 모두 60% (Group 1; 12/20 례, Group 2; 15/25 례)로 감소하는 경향을 나타내었다. 대장선종의 마리당 발생개수에 있어서는 C57BL/6J- *Apc*$^{Min/+}$계 수컷 이형접합체 형질전환 마우스에 AIN-76A 정제사료만을 투여한 대조군은 1.40±0.24 (100%)에 비하여 I3C 저농도 투여 실험군 (Group 1; 0.85±0.23; 61%, $P<0.01$), 그리고 I3C 고농도 투여 실험군 (Group 2; 1.32±0.29; 94%)의 순으로 감소하였다. 선종의 크기별 종양의 발생개수의 분포에 있어서 I3C 저농도 투여 실험군에 있어서는 선종의 크기가 3mm 이하의 수가

현저하게 감소하였다. C57BL/6J-*Apc*$^{Min/+}$ 계 수컷 이형접합체 형질전환 마우스에 AIN-76A 정제사료만을 투여한 대조군의 부위별 소장선종의 발생개수는 십이지장부위를 제외하고 각 군에서 유의한 변화는 관찰되지 않았다. 십이지장 종양의 발생개수에서만 I3C 저농도 투여 실험군 (Group 1; 3.11±0.85)이 대조군 (Group 3; 1.48±0.35) 및 I3C 고농도 투여 실험군 (Group 2; 1.56±0.47)에 비하여 유의성 있게 증가하였다 ($P<0.05$). 따라서 I3C은 소장에서는 암예방 효과가 뚜렷하지 않으나, 대장에 대한 암예방 효과가 있을 것으로 생각된다. 소장 및 대장을 제외한 간장, 신장, 비장, 심장, 폐, 그리고 위 등의 기타 장기에서의 조직병리학적 변화는 관찰되지 않았다. 소장 및 대장의 종양은 선종(polyps)으로 관찰되었다.

지난 10 여년간 형질전환 및 유전자결핍 실험동물의 종류가 기하 급수적으로 증가하여 이용되고 있다. 가족성 대장 선종성 용종증(FAP)의 대표적인 모델로 이용되고 있는 C57BL/6J- *Apc*$^{Min/+}$계 수컷 이형접합체 형질전환 마우스를 사용하여 배추나 양배추의 주요 성분인 Indole-3-carbinol (I3C)의 대장암 예방효과가 있는지를 검색하여 본 결과 AIN-76A 정제사료만을 투여한 대조군의 대장선종의 발생률 84%에 비하여 I3C 100 및 300ppm 을 투여한 실험군에 있어서 각군 모두 60%로서 감소하는 경향을 나타내었으며, 대장선종의 마리당 발생개수에 있어서는 대조군의 1.40±1.041 를 100%로 환산하였을 경우 I3C 저농도 및 고농도 투여 실험군에서는 각각 약 61%와 94%를 나타내어 감소하였다. 특히 대장 선종의 크기별 분포에 있어서 선종의 크기가 3mm 이하의 수가 현저하게 감소하였다. 따라서 저농도 I3C의 투여는 실험적 유전성 가족성 대장 선종성 용종증 모델에 있어서 어느 정도 암예방효과가 있는 것으로 생각된다. 그러나 소장 선종의 발생에는 별 영향이 없는 것으로 생각된다. 그러나 본 실험에 사용된 C57BL/6J- *Apc*$^{Min/+}$계 수컷 이형접합체 형질전환 마우스는 실험 개시 시점이 7 내지 8 주령이 경과하여 이미 태생기부터 소장 및 대장의 선종의 발생이 진행되어 온 것을 감안하고, 특히 비스테로이드계 항염증 소염제 (NSAIDS)와 같은 강력한 COX-2 억제제가 아님을 고려하면, 상당한 선종의 발생을 억제할 수 있는 가능성이 매우 높다고 생각한다. 또한 이제까지 배추나 양배추 성분의 복합성분들에 대한 실험적 대장암 모델에서의 촉진효과 등에 대한 보고들이 있어 온 점을 고려할 때 위암 (Kim 등, 1994), 간암 (Kim 등, 1994), 유방암 (Grubbs, 등, 1995; Bradlow 등, 1995)에 대한 예방효과가 있을 것으로 생각한다.

앞으로 이러한 종양조직내에서의 COX-2 및 iNOS mRNA 와 단백질의 발현정도를 분자병리학적으로 연구중에 있으며, 향후 십자화과식물 성분인 indole-3-carbinol 이 마우스뿐 만 아니라 랫드의 화학발암물질에 의한 대장종양에 대한 억제효과 있는지 연구할 필요가 있다. Min 마우스와 같은 형질전환 (유전자결핍: knockout) 실험동물을 이용한 새로운 중기 발암성 시험법의 확립을 통한 각종 환경유해물질의 발암성 유무 및 COX-2 억제작용이 있는 식품인자의 암예방 후보물질을 체계적으로 검색하는 데 유용하게 활용될 수 있을 것으로 생각한다.

Legends for figures

Figure 1. The chemical structure of indole-3-carbinol (I3C).

Figure 2. Experimental protocol for the knockout *Min*/+ mouse carcinogenesis model.

Figure 3. Experimental protocol for AOM-induced colon carcinogenesis model.

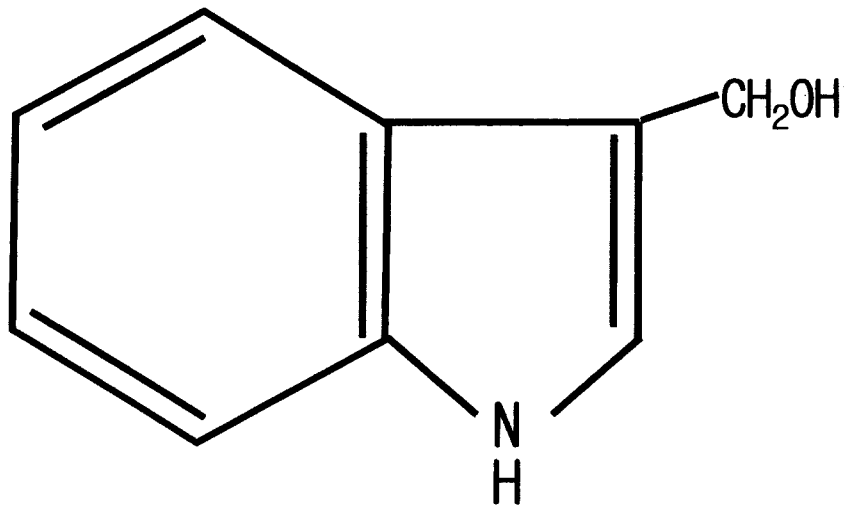
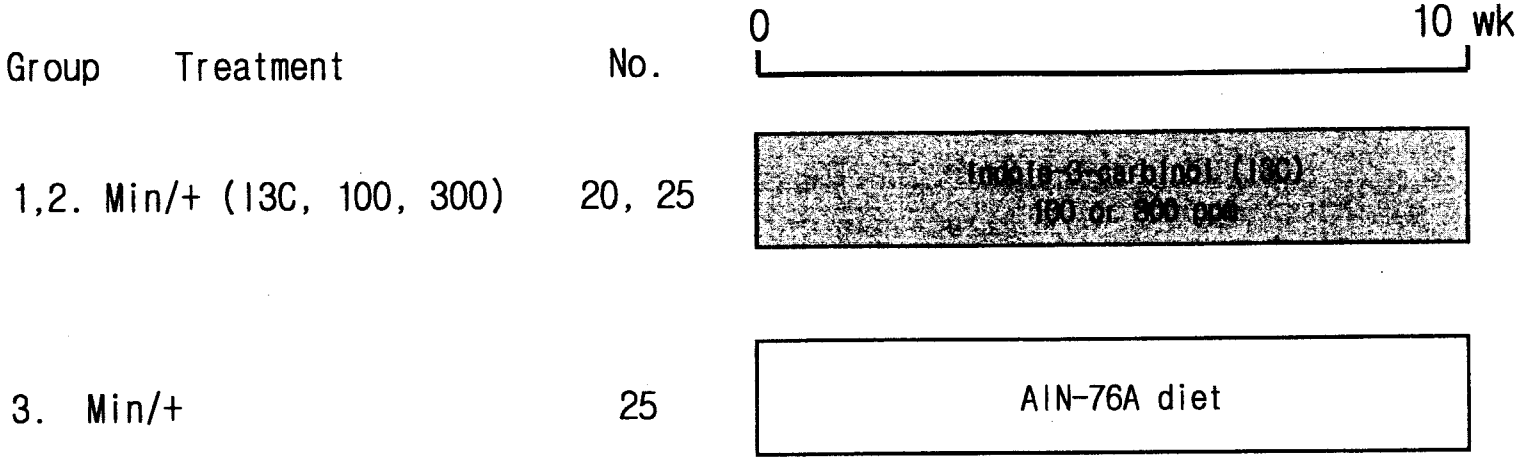
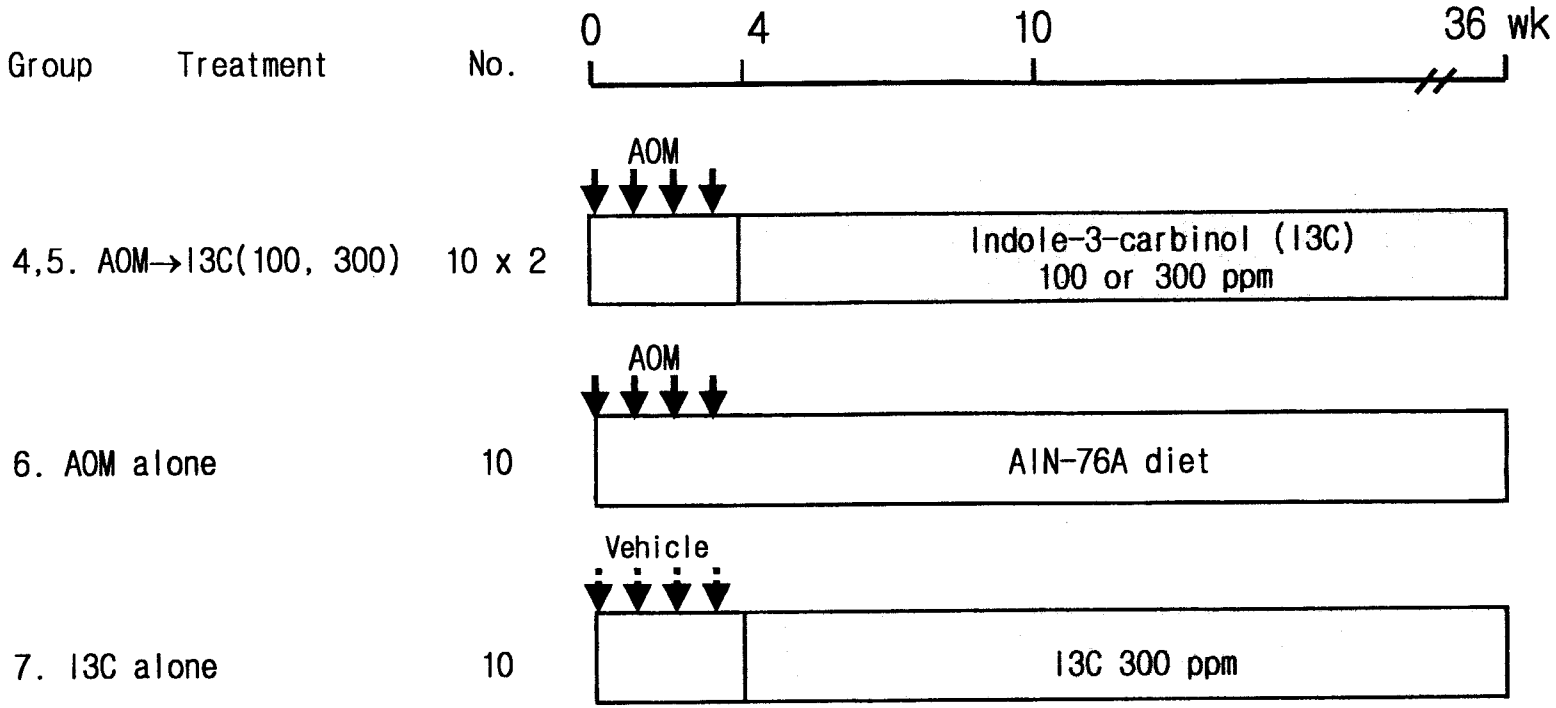


Figure 1. Structure of indole-3-carbinol (I3C)



Animals: Seventy male C57BL/6J-*Apc*$^{Min/+}$ mouse, 6-wk-old mice
Min/+ : C57BL/6J-*Apc*$^{Min/+}$ mice (The Jackson Lab., Bar Harbor, ME, USA)
I3C : Diet containing indole-3-carbinol (100 or 300 ppm) in AIN-76A (Harlan Teklad Research Diet, USA) diet was given for 10 wk.

Figure 2. Experimental protocol for the knockout *Min/+* mouse carcinogenesis model



Animals: Forty male normal wild type C57BL/6J-*Apc*$^{Min+/+}$ littermates (*Min+/+*) of 6-wk-old mice (The Jackson Laboratory, Bar Harbor, ME, USA)

I3C : Diet containing Indole-3-carbinol (100 or 300 ppm) in AIN-76A diet (Harlan Teklad Research Diet, USA) was given for 32 wk from week 4.

AOM : Azoxymethane (5mg/kg bw, s.c. inj.) was s.c. injected four times for 4 week at the start.

Figure 3. Experimental protocol for AOM-induced colon carcinogenesis model