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■ Brief Communication ■

Modifying Effect of Indole-3-carbinol on Azoxymethane-induced Colon Carcinogenesis

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Indole-3-carbinol (I3C), one of naturally occurring main components in cauliflower vegetables, is supposed to have a chemopreventive potential in experimental animals and humans. This study was investigated to examine chemopreventive effect of I3C on colon carcinogenesis induced by azoxymethane (AOM) using C57BL/6J mice. Mice were divided into three groups (10 or 9 mice/group). All mice were subcutaneously injected with AOM (5 mg/kg body weight, four times at weekly interval). After AOM treatment, animals of group 1 were fed by AIN-76A pellets as a basal diet. Animals of groups 2 and 3 were given I3C containing diets (100 and 300 ppm in diets, respectively) for 6 weeks until sacrifice. All mice were sacrificed at week 10 and the aberrant crypt foci (ACF) of the colonic mucosa were assessed after staining with methylene blue. Total numbers of ACF/colon in group 2 (10.1±5.1) or group 3 (10.6±5.3) were decreased compared to the values of group 1 (14.4±10.2). Among numbers of ACF formation, 5, 7, 8 and 10 ACF in group 2 and 3 were greatly different those of group 1. Total numbers of aberrant crypts (AC)/colon of group 2 (20.1±10.1) or group 3 (22.0±10.9) were decreased compared to the value of group 1 (33.7±24.7). Taken together, it suggests that I3C treatment may retard mouse colon carcinogenesis even after administration of AOM.

Key Words: Indole-3-carbinol (I3C), C57BL/6J mice, Azoxymethane (AOM), Aberrant crypt foci (ACF), Aberrant crypt (AC)

Several compounds naturally occurring in vegetables and fruits has many advantages for cancer prevention in human uses (Kelloff et al., 2000). It has been reported that consumption of cruciferous vegetables such as cabbages and broccoli could be associated with cancer chemopreventive effects in humans (Steinmetz and Potter, 1996).

Indole-3-carbinol (I3C), one of naturally occurring main components in cauliflower vegetables, has been tested as a potential chemopreventive factor in humans as well as experimental animals. It is produced endogenously from naturally occurring glucosinolates, whenever they are crushed or cooked, and acid environment of gut facilely converts it into a range of polyaromatic indolic compounds (Broadbent and Broadbent, 1998).

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It has been reported that I3C has considerable potential as a prophylactic anticancer agent against some neoplasms such as in colon (Wargovich et al., 1996), liver (Dashwood et al., 1989), mammary gland (Grubbs et al., 1995; Kang et al., 2001) and uterus (Kojima et al., 1994) when it is applied prior to or concurrently with exposure to the carcinogen. In colon, I3C and its metabolites treatment inhibited colonic aberrant crypts (Guo et al., 1995) and colonic neoplasm (Wargovich et al., 1996). And As chemopreventive effect of I3C is believed to be associated with pathways of cell cycle arrest (Cover et al., 1998; Cover et al., 1999), disruption of cyclin E protein processing (Nguyen et al., 2008) and apoptosis of tumor cells (Bonnesen et al., 2001), it is anticipated that I3C could block carcinogenesis even after initiation.

However, it has been reported that post-initiation treatment of I3C enhaces promoting potetional in liver (Kim et al., 1997; Oganesian et al., 1999), mammary gland (Kang et al., 2001). As I3C and its several metabolites act to

induce aryl hydrocarbon receptor mediating response (Bjeldanes et al., 1991; Jellinck et al., 1993), there may be a carcinogenic chance under specific conditions, especially when initiated cells or tumor cells are growing. These concepts have led to consider time points of carcinogen exposure and cellular circumstances when chemicals are applied.

As there are still little understandings about post-initiation action of I3C on colon carcinogenesis, we investigated modifying effect of I3C in azoxymethane (AOM)-induced colon carcinogenesis model.

Twenty-nine, 5 week-old, male C57BL/6J mice were purchased from the Jackson Laboratory, USA. The animals were housed in polycarbonated cages with hardwood chips, in a room with a 12 hour light-dark cycle and controlled humidity and temperature (23±2℃, 55±10% RH). They were allowed free access to distilled water and pellet chow basal diets. I3C was obtained from the Sigma Chemical Co., St. Louis, MO, USA. AIN-76A purified powdered and pellet diets were obtained from the Harlan Teklard Research Diets, USA. C57BL/6J mice were divided into three groups (number of animal in group 1 as 10, group 2 as 9, group 3 as 10). The animals were subcutaneously injected with AOM (5 mg/kg body weight, four times at weekly interval) from 6 week-old of age for induction of colon carcinogenesis.

Animals of group 1 were placed on the basal diets of AIN-76A pellets as a control. Animals of group 2 and 3 were given I3C containing diets (100 or 300 ppm, respectively) for 6 weeks until sacrifice. All mice were sacrificed at the end of the experiment at week 10.

The colon was inflated with saline after ligation, longitudinally cut into and stretched out the filter papers. For measuring ACF and AC of the colon (groups 1, 2 and 3), colon samples were stained with methylene blue and were counted by microscope as reported previously (Bird and Good, 2000). After counting of ACF, colon was fixed in 10% neutral phosphate buffered formalin. The fixed colon were trimmed, processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E), and carried out histopathological examination under microscope.

Statistical analyses were performed using the JMP program (SAS Institute, Cary, NC). The Duncan's new multiple range test was employed for comparison of ACF and AC data between control and treated groups. For all comparisons, probability values less than 5% ($P<0.05$) were considered to be statistically significant.

Total numbers of ACF/colon in groups 2 or 3 tended to decrease compared to group 1. Total numbers of ACF/colon in group 2 (10.1±5.1) or group 3 (10.6±5.3) were decreased compared with the values in group 1 (14.4±10.2). Among numbers of ACF formation, 5, 7, 8 and 10 ACF in group 2 and 3 were greatly different that of group 1. Total numbers of aberrant crypts (AC)/colon of group 2 (20.1±10.1) or group 3 (22.0±10.9) decreased compared with the value for group 1 (33.7±24.7) (Table 1).

Representative figures for ACF were presented in Fig. 1 as (A) colonic ACF from the animal treated with azoxymethane (AOM) and (B) colonic ACF from the animal treated with AOM and indole-3-carbinol.

Histopathological examination showed that aberrant crypt formation in colon. However, there were no morphological differences of ACF formation between control and I3C treatment groups (data not shown).

Table 1. The number of aberrant crypt foci (ACF) and aberrant crypt (AC) in C57BL/6J mice treated with indole-3-carbinol (I3C)

Group	No of animal	ACF										Total ACF	Total AC
		1	2	3	4	5	6	7	8	9	10		
AOM alone	10	4.8±4.0	9.2±6.6	9.9±10.0	2.0±3.4	3.5±4.1	0	0.7±2.2	1.6±3.4	0	2.0±4.2	14.4±10.2	33.7±24.7
AOM+I3C 100 ppm	9	3.6±2.4	7.8±4.4	6.3±4.6	1.8±2.9	0	0.7±2.0	0	0	0	0	10.1±5.1	20.1±10.1
AOM+I3C 300 ppm	10	3.6±2.7	8.8±4.0	3.6±3.7	4.4±4.8	1.0±2.1	0.6±1.9	0	0	0	0	10.6±5.3	22.0±10.9

[a] Data of the ACF and AC represent mean ± SD.
C57BL/6J mice were given diets containing indole-3-carbinol (100 or 300 ppm) in the AIN-76A.

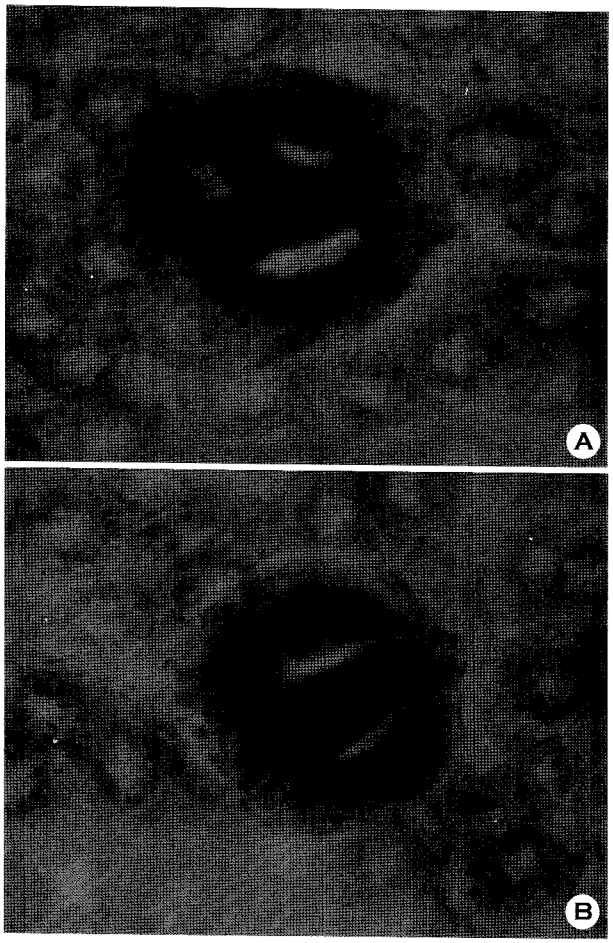

Fig. 1. Aberrant crypt foci (ACF) in the colonic mucosa of rats. (A) Colonic ACF from the animal treated with azoxymethane (AOM) alone, (B) Colonic ACF from the animal treated with AOM and indole-3-carbinol 300 ppm. Methylene blue-stained whole mount, ×400.

As it seemed that consumption of I3C after carcinogen treatment induced tumor promotion in colon (Pence et al., 1986), liver (Kim et al., 1994) and mammary gland (Kang et al., 2001), it is considered that modifying effect of I3C may be different at the time of carcinogen exposure and types of carcinogens (Dashwood, 1998; Kang et al., 2000).

As there is still controversy of modifying effect of I3C depending on the stage of tumor formation, it is an important approach to test tumor-inhibiting effect of I3C after initiation. In this study, post-initiation treatment of I3C decreased the formation of ACF, colonic preneoplastic lesions. These results suggest that I3C may have tumor-inhibiting action on the colon carcinogenesis in mice after initiation.

ACF is one of the earliest visible event in carcinogen-induced colorectal cancer models (Fenoglio-Preiser and Noffsinger, 1999) and the growth, morphological and molecular features of ACF support the contention that ACF are putative preneoplastic lesions and the ACF system is used extensively to identify modulators of colon carcinogenesis (Bird and Good, 2000).

AOM is an organotropic colon carcinogen that is commonly used to induce colon tumors in rodents (Papanikolaou et al., 1998). And cyclin D1 and Cdk4 expression were markedly increased in preneoplastic lesions and in adenomas isolated from AOM-treated mice, and suggest that overexpression of cyclin D1 and Cdk4 occurs early in the AOM-induced mouse colon tumorigenesis and may contribute to tumor progression in this model (Wang et al., 1998). So, AOM-induced mouse colon carcinogenesis model is to test how dietary factor could affect the rate of ACF formation. In this study, cell cycle arrest by I3C treatment may be associated with inhibition of AOM-induced colon carcinogenesis.

It is reported that AOM-induced ACFs had diffuse loss of hexosaminidase activity, variable depletion of mucin, and the ACF assay may be a useful method to improve the identification and characterization of xenobiotic-induced changes in colonic mucosal crypts (Whiteley et al., 1996). In this study, among numbers of ACF formation, 5, 7, 8 and 10 AC in group 2 and 3 were greatly different those of group 1. As higher number of AC may be correlated with colon cancer, I3C treatment retard tumor inhibition associated with the decrease of larger AC.

As dysplastic ACF which were characterized by fast-growing crypts with altered control of beta-catenin (Paulsen et al., 2001), and beta-catenin is frequently mutated in chemically-induced colon tumors (Takahashi et al., 1998), it seems that dysplastic ACF may be major sources of colon tumors. However, it is not clear that I3C may affect these ACF. As it was suggested that the majority of intestinal tumors in Min mice were initiated relatively early in life (Shoemaker et al., 1995), it is possible to test how dietary factor could affect the rate of tumor initiated by targeting the gatekeeper from of the APC gene. As reported that I3C may be a potential chemopreventive agent for Min mice colon cancer model (Kim et al., 2003), further studies

will be warranted to investigate I3C action on dysplastic ACF, especially associated with tumor suppressor gene(s). And as I3C showed inhibition of colon cancer cells with combination of other phytoestrogens (Nakamura et al., 2009; Kronbak et al., 2010), it will be a good approach and may provide combination therapies for inhibition of colon carcinogenesis.

Taken together, it suggests that I3C treatment may retard mouse colon carcinogenesis even after administration of AOM.

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