RESEARCH COMMUNICATION

Modifying Effect of Diallyl Sulfide on Colon Carcinogenesis in C57BL/6J-Apc[Min/+] Mice

Jin Seok Kang[1], Tae Myoung Kim[2], Tae Jin Shim[2], Elsayed I Salim[3], Beom Seok Han[4], Dae Joong Kim[2]*

Abstract

   Diallyl sulfide (DAS), a flavoring compound derived from garlic, is considered to have cancer chemopreventive potential in experimental animals and humans. This study was designated to examine possible chemopreventive effects of DAS on colon carcinogenesis using genetically engineered transgenic Apc[Min/+] mice, a well-established animal model for familial adenomatous polyposis (FAP) and sporadic colorectal cancer. Male C57BL/6J-Apc[Min/+] mice were divided into three groups. Animals of group 1 were placed on the basal diet (AIN-76A) as non-treated controls. Animals of groups 2 and 3 were given DAS-containing diets (in doses of 100 and 300 ppm, respectively). All mice were sacrificed at the end of week 10 of the experiment. Histopathological investigation revealed that the incidence of colonic polyps was decreased dose-dependently by 19% (13/16) in group 2 and by 32% (13/20) in group 3 compared to the 100% incidence (10/10) in group 1. The multiplicity of colonic polyps per mouse was also slightly decreased by DAS treatment (1.88±0.35 in group 2 and 1.63±0.36 in group 3) compared to 2.00±0.39 in group 1. On the other hand, there were no significant differences in the numbers of total polyps per mouse in the small intestine between the groups. Taken together, we suggest that DAS may exert promising inhibitory effects on colon carcinogenesis in the transgenic Apc[Min/+] mice.

Key words: Diallyl sulfide (DAS) - C57BL/6J-Apc[Min/+] - multiple intestinal neoplasia (Min) - chemoprevention

Introduction

   Several naturally occurring compounds particularly those found in fruits and vegetables have many cancer chemopreventive properties in human and experimental animals (Kelloff et al., 2000). Previous our studies proved that consumption of vegetables had been shown to have cancer chemopreventive effects on colon cancer (Kang et al., 2000; Kim et al., 2003). Of these compounds, garlic and organosulphur compounds have been reported to be associated with reduced cancer risk in human (Khanum et al., 2004). When garlic is cut, chopped or crushed, it is metabolized into diallyl sulfide (DAS) and other compounds (Shukla and Kalra, 2007). Garlic metabolites has been shown to exert its anticarcinogenic effects through diverse mechanisms, including free radicals scavenging, increasing the activities of enzymes such as glutathione S-transferase and catalase, inhibition of cytochrome *p450* 2E1 as well as up-regulation of the

DNA repair mechanisms and prevention of chromosomal damage (Khanum et al., 2004).

   The protective effects of DAS and its related compounds against carcinogenesis have been shown in stomach, esophagus, mammary glands, breast, skin and lungs of experimental animals (Moriarty et al., 2007). Also it has been reported that DAS and its related compounds have considerable inhibitory potential on colonic aberrant crypt foci (Wargovich et al., 1996), colonic neoplasms (Herman-Antosiewicz and Singh, 2004) as well as on the migration and invasion of colon cancer cells *in vitro* (Lai et al., 2011).

   Familial adenomatous polyposis (FAP) is an autosomal, dominantly inherited disease. Patients with FAP inherit adenomatous polyposis coli (APC) genetic mutations develop numerous dysplastic aberrant crypt foci (ACF) and progressively develop numerous colorectal tumors (Kinzler and Vogelstein, 1996).

   Multiple intestinal neoplasia (Min) mice have a

[1]Department of Biomedical Laboratory Science, Namseoul University, Cheonan, [2]College of Veterinary Medicine & Research Institute of Veterinary Medicine, Chungbuk National University, Cheongju, [3]Department of Zoology, Tanta University, Tanta, Egypt, [4]Division of Toxicopathology, Hoseo Toxicology Research Center, Hoseo University, Asan, Korea *For correspondence: kimdj@cbu.ac.kr

germ-line nonsense mutation in the *APC* gene and spontaneously develop early multiple polyps in the small and large intestines (Su et al., 1992). As a result to this genetic mutation, these mice predisposes to spontaneous intestinal cancer throughout the entire intestinal tract at an early age (Moser et al., 1990). So, they are considered to be a good animal model to study human FAP and sporadic colorectal cancer on the experimental levels.

As the chemopreventive effect of DAS was shown to be associated with cell cycle arrest and apoptosis pathways in tumor cells (Sriram et al., 2008), we thought that DAS could block carcinogenesis after initiation stage of carcinogenesis. To test this possibility, we investigated the chemopreventive effect of DAS on colon carcinogenesis using $Apc^{Min/+}$ mice.

Materials and Methods

Chemicals

DAS was obtained from the Sigma Chemical Co., St. Louis, MO, USA. The structure of DAS is illustrated in Figure 1. Its purity was 100% with no detectable inorganic compounds when subjected to ion chromatography (IC) or inductive coupled plasma mass spectrometry (ICP–MS).

Animals

Seventy, 7 week-old, male transgenic heterozygous $C57BL/6J-Apc^{Min/+}$ mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, USA. The animals were housed in polycarbonated cages with hardwood chips, in a room with a 12 hour light-dark cycle and a controlled temperature of 23±2°C and humidity of 55±10% RH. They were allowed free access to distilled water and AIN-76A pellet chow basal diets obtained from the Harlan Teklard Research Diets, USA.

Treatment

$C57BL/6J-Apc^{Min/+}$ mice were divided into three groups. Animals of group 1 (10 mice) were placed on the basal diet of AIN-76A pellets as a non-treated control group. Animals of groups 2 (16 mice) and 3 (20 mice) were given DAS (100 or 300 ppm respectively in diet) for 10 weeks until sacrifice. All mice were sacrificed at the end of the experimental week 10.

Macroscopic scoring of intestinal polyps

All animals were sacrificed under excess ether anesthia. The whole intestines were removed, inflated with saline after ligation, longitudinally cut through its median axis, then stretched flat on filter papers. The small and large intestines were distinguished from each other and fixed separately in 10% phosphate buffered formalin at 4°C for at least 48 hours.

The numbers and sizes of gross tumors and polyps in formalin-fixed intestines were measured witht the aid of an IBAS automatic image analyzer (Kontrons Co. Ltd., Germany).

Histopathological examination

The tumors were then processed, embedded in paraffin, sectioned at 4 μm, and routinely stained with hematoxylin and eosin (H&E) for microscopic histopathological examination.

Statistical analyses

Intestinal tumor incidences were compared by the Likelihood ratio Chi-squared test. The numbers of tumors per mouse (multiplicities) were analyzed by the Duncan's *t-test* (StatView, ver.5, Abacus Corp., CA, USA).

Results

Incidence and multiplicties of colonic polyps

The incidence of colonic polyps was 100% (10/10) in group 1. DAS treatment has decreased the incidence of colonic polyps into 81% (13/16) in group 2 and to 68% (13/20) in group 3. The reduction was by 19% and 32% in groups 2 and 3 respectively, compared to the data of group 1. The number of colonic polyps per mouse in group 1 was 2.00±0.39 while that of group 2 was 1.88±0.35 and group 3 was 1.63±0.36, also showed a slight decrease compared to group 1 (Table 1).

Effect on small intestinal polyps

There were no differences in the number of total polyps per mouse in the small intestine between the groups (Table 2). However, some differences occurred in the distribution of polyps in different intestinal regions. The numbers of duodenal polyps per mouse was higher in groups 2 (6.75±1.09) and 3 (4.68±0.62) as compared to that of group 1 (2.50±0.57) (p<0.01). Also and the numbers of jejunal polyps per mouse was higher in group 2 (21.00±2.88) as compared to that of group 1 (14.90±2.24) (p<0.01). However, there were no differences in the numbers of ileac polyps and total numbers of small intestine per mouse among the groups.

Histopathological examination

Histopathological examination revealed that all tumors were intestinal polyps. There were no differences in polyps' pathology between control and DAS-treated groups. On the other hand, there were no histopathological differences in the liver, kidneys and spleen or in any other vital organs of mice between the groups (data not shown).

Table 1. Incidences and Multiplicities of the Colonic Polyp of C57BL/6J-Apc$^{Min/+}$ Mice Treated with Diallyl Sulfide (DAS)

Group	Treatment	No. of mice	Incidence (%)	Multiplicity[a]
1	Control	10	10/10 (100)	2.00±0.39
2	AS 100 ppm	16	13/16 (81)	1.88±0.35
3	AS 300 ppm	20	13/20 (68)	1.63±0.36

[a]Data of the colonic polyps represent means±SE; C57BL/6J-Apc$^{Min/+}$ mice were given AIN-76A diets containing 0, 100 or 300 ppm DAS.

Table 2. Effects of Diallyl Sulfide (DAS) on Small Intestinal Polyps of C57BL/6J-Apc$^{Min/+}$ Mice

Group	Treatment	No. of mice	Small intestine			
			Duodenum	Jejunum	Ileum	Total
1	Control	10	2.50±0.57	14.90±2.24	36.70±5.50	54.10±7.50
2	AS 100 ppm	16	6.75±1.09[*a]	21.00±2.88[*]	31.63±3.37	59.40±4.90
3	AS 300 ppm	20	4.68±0.62[*]	17.30±1.39	32.95±2.50	54.95±3.01

Mean of the number of polyps ± SE; [*]Significantly different from the value of control group at p<0.01; [a]Significantly different from the value of DAS 300 ppm group at p<0.01; C57BL/6J-ApcMin/+ mice were given diets containing DAS (100 or 300 ppm) in the AIN-76A.

Discussion

As Apc$^{Min/+}$ mice are good bioassay for the FAP, it is possible to test how dietary factors such as DAS could affect the rate of tumor initiation and progression by targeting the gatekeeping form of the *APC* gene. The *APC* gene is a tumor suppressor gene, which regulates the cell cycle division by keeping cells from growing and dividing too fast or in an uncontrolled way. The germline mutated APC protein cannot suppress the cellular overgrowth that leads to the formation of polyps, which can become cancerous in the future (Groden et al., 1991).

It was suggested that the majority of intestinal tumors in Min mice were initiated at relatively early age (Shoemaker et al., 1995). Thus the present study is interesting approach to test DAS tumor-inhibitory effects at early initiation stages of carcinogenesis.

In this study, the incidences and multiplicities of colonic polyps in DAS treatment groups highly tended to decrease compared to the data of the control group. In particular, the incidences were inhibited dose-dependently by 19% and 32% in the mice treated by 100 and 300 ppm DAS respectively. Although this inhibition did not reach statistical significant values probably due to the few numbers of mice tested, we assume that this inhibition could be an indicator for a promising inhibitory effect of DAS on FAP and on early colon carcinogenesis in both the initiation and post initiation stages. This assumption warrants further studies for confirmation.

Patients with FAP inherit *APC* mutations and develop early numerous dysplastic ACF, some of which progress into cancer after acquiring other genetic mutations such as K-*ras* (Kinzler and Vogelstein, 1996) and altered control of beta-catenin (Paulsen et al., 2001). The loss of functional APC protein results in the accumulation of beta-catenin, which was reported to contribute to neoplastic transformation by causing accumulation of cyclin D1 (Tetsu and McCormick, 1999). Beta-catenin is frequently mutated in chemically-induced colon tumors (Takahashi et al., 1998). Previously, DAS was shown to inhibit the total numbers of colonic ACF (Wargovich et al., 1996). Further studies are warranted on the modifying effects of DAS on beta-catenin mutations in colon carcinogenesis.

The cell cycle-related genes are known to be amplified in the intestinal adenomas of Min mice and in human FAP patients with concurrent overexpression of cyclin D1 and cyclin-dependent kinase 4 (Cdk4) (Zhang et al., 1997). This was also evident in the chemically-induced mouse colon carcinogenesis (Wang et al., 1998) and in human colorectal carcinomas (Leach et al., 1993). Previously, it was shown that normally-appearing bowel mucosa of Min mice had decreased numbers of apoptotic cells among enterocytes (Boolbol et al., 1996). Also, DAS treatment was shown to induce cell cycle arrest and apoptosis in tumor cells (Sriram et al., 2008). Therefore, the present decrease in the incidences and multiplicities of colonic polyps by DAS could be explained to be associated with increased apoptosis levels in the colons of Min mice induced by DAS. This needs further confirmation.

Taken together, we conclude that DAS treatment showed a great tendency to decrease the incidences and multiplicities of colonic polyps in the transgenic Apc$^{Min/+}$ mice, suggesting that DAS might be a promising chemopreventive agent for colon carcinogenesis in FAP patients.

Acknowledgements

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