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Effect of Dietary Selenium on the Colon Carcinogenesis in Male ICR Mice

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ABSTRACT - Selenium is an essential micronutrient for normal body function and functions as an essential constituent of selenoproteins. This study was carried out to investigate effect of selenium on the formation of colonic aberrant crypt foci (ACF) and tumor formation in a mouse model. Five-week old ICR mice were acclimated for one week and fed different selenium diet (0.02, 0.1, and 0.5 ppm) for 12 weeks. Animals received three intraperitoneal injections of azoxymethane (10 mg/kg B.W. in saline for 3 weeks), followed by 2% dextran sodium sulfate in the drinking water for a week. There were four experimental groups, including a normal control group and three different selenium levels groups. After sacrifice, the total numbers of aberrant crypt (AC) and ACF were measured in the colonic mucosa after methylene blue staining. The number of tumors was noted for tumor incidence. Liver selenium concentration was measured using ICP-AES method. Gutathione peroxidase (GPx) activity was determined using a GPx assay kit in the liver and colon. TUNEL assay and proliferating cell nuclear antigen (PCNA) staining were performed to examine the cell apoptosis and cell proliferation, respectively. Immunohistochemistry of β-catenin was also performed on the mucous membrane tissue of colon. The activity of GPx in the liver and colon was decreased in the selenium-deficient diet group while it was increased in the selenium-overloaded diet group. Apoptotic positive cells were increased in the selenium-overloaded diet group but decreased in the selenium-deficient diet group. PCNA staining area was decreased in the selenium-overloaded diet group. In addition, the β-catenin protein level in the selenium-deficient diet group was increased but decreased in the selenium-overloaded diet group. These results indicate that dietary selenium might exert a modulating effect on colon cancer by inhibiting the development of ACF and colon tumor formation in this mouse model.

Key words : azoxymethane (AOM), dextran sodium sulfate (DSS), colon cancer, aberrant crypt foci, aberrant crypt, selenium

In developed countries, the colon is a common site for malignancies of the gastrointestinal tract, which are a leading cause of death. The progressive introduction of Western dietary habits, especially increased fat intake and reduced carbohydrate and dietary fiber intake, has increased the incidence of colorectal cancer (CRC) and related deaths[1)]. CRC is one of the most frequent cancers worldwide. CRC is the third leading cause of cancer-related death in men and women in the United States[2)]. In Korea, the incidence and mortality of CRC have gradually increased in the last decade, becoming the fourth leading cause of cancer deaths[3)].

Since Aberrant crypt foci (ACF) were first reported in the colons of carcinogen-treated C57BL/6J of CF1 female mice[4)], ACF can be used as the endpoint in CRC development, because aberrant crypts are postulated to be the earliest identifiable potential precursors of CRC in rodents and humans. The analysis of ACF may facilitate the study of the early pathological and molecular changes that precede the development of an adenoma to CRC[5,6)]. These ACF are characterized by crypts with altered luminal openings, thickened epithelia and being larger than the adjacent normal crypts[7)].

Azoxymethane (AOM) is an indirect and genotoxic carcinogen requiring metabolic activation of CYP2E1 to exert its carcinogenic action[8)]. The most commonly used mouse model employs dextran sodium sulfate (DSS), which is a non-genotoxic carcinogen in the colon[9,10)]. However, CRC development in the DSS colitis model typically requires a relatively long exposure period or cycles of DSS administration. Furthermore, the incidence and/or multiplicity of induced tumors is relatively low[11)]. A novel colitis-related mouse CRC model initiated with AOM and promoted using DSS was developed to obtain a better understanding of the pathogenesis of CRC[12-14)].

Selenium, which may prevent carcinogenesis through several biochemical pathways, is an essential trace element found in cereals, wheat, dairy products, meat and fish[15)].

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Selenium has been found to reduce the incidence and progression of cancer in animals exposed to carcinogens[16,17], and low serum or plasma selenium concentrations have been found to be associated with increased CRC[18]. Possible biological mechanisms for this association include repair and prevention of oxidative damage, intracellular signaling, activation of thyroid hormone, regulation of immune response and enhanced apoptosis[19-21].

β-catenin is universally localized to the cytoplasm and/or nucleus[22]. The altered expression of β-catenin was reported in inflammation-related colonic cancer in rodents[12]. Extensive fragmentation of nuclear DNA that generates a large number of DNA double-strand breaks is one of the most characteristic events of apoptosis[23,24]. Proliferating cell nuclear antigen (PCNA), which is a nonhistone nuclear protein required for DNA synthesis, acting as the auxiliary protein of DNA polymerase α, has been described as an indicator of cell proliferation[25]. It is obvious that there is a need for new histological markers such as PCNA and TUNEL for more objective assessment of the premalignant stage of colon and more useful aid in estimation of the future neoplastic progression.

The aims of this study were to elucidate how dietary selenium influence colon carcinogenesis induced by AOM followed by DSS in mice by determining several cell proliferation indices and ACF.

Materials and Methods

Materials

Azoxymethane (AOM) was purchased from the Sigma Chemical Company (St Louis MO, USA) and dextran sodium sulfate (DSS) (molecular weight 36,000~50,000) was manufactured by MP biomedicals (Solon, OH, USA).

Animals

Male ICR mice (5 weeks old) were obtained from Central Lab. Animal Inc (SLC Inc., Shizuoka, Japan), housed in polycarbonate cages (5 mice/cage). The temperature and relative humidity were maintained at 20 ± 2°C and 50 ± 20%. Light and dark cycles were at 12 h each. Mice were allowed access to AIN-93G purified rodent diet (Dyets, Inc., Easton Avenue, Bethlehem, USA) and water was provided ad libitum. The animal experiments were conducted in accordance with "Guide for care and use of Laboratory animals" of Chungbuk National University. After one week of acclimatization, the animals were then taken off chow feed, and placed on one of purified diets containing modified levels of selenium. During the experimental period, weekly body weights and feed consumptions were recorded.

Experimental designs

Twenty mice were assigned to each treatment group, while six mice to normal control group. The experimental period was 12 weeks. There were normal control group, AOM/DSS positive control group and two treatment groups (Fig. 1). The mice were fed AIN-93G purified rodent diet with the concentration of 45 ppm iron. Additionally, the mice in two treatment groups were fed the selenium-deficient diet (LSe, 0.02 ppm selenium) or selenium-overloaded diet (HSe, 0.5 ppm selenium), whereas the mice in the normal control and AOM/DSS positive control groups were fed the normal selenium diet with the concentration of 0.1 ppm selenium.

Experimental diets

The AIN-93G purified rodent diet contained 20% casein, 0.3% L-cystine, 39.7% cornstarch, 13.2% dyetrose, 7% soybean oil, 0.0014% t-butylhydroquinone, 5% cellulose, 3.7% mineral mix, 1% vitamin mix, 0.25% choline bitartrate and 0.9% ferric citrate (Table 1). Normal selenium, selenium-deficient diet (LSe) and selenium-overloaded diet (HSe) contained 0.01%, 0.002% and 0.05% Na selenate, respectively.

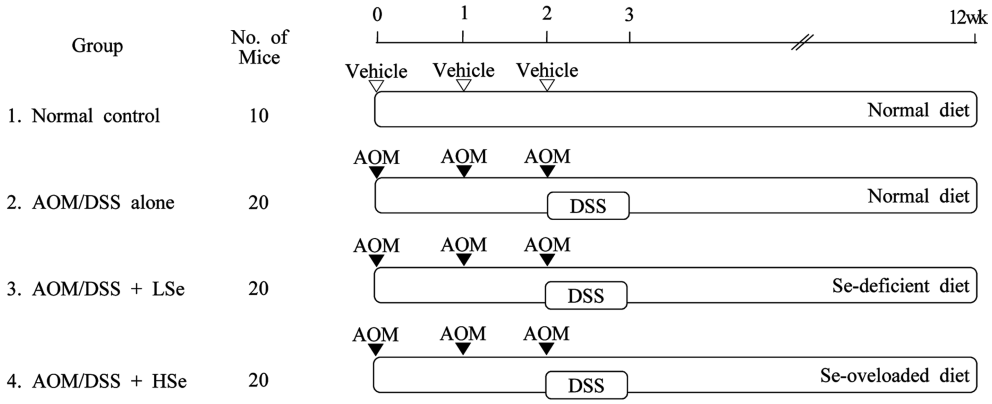

Fig. 1. Experimental design for colon carcinogenesis in mice. AOM : Azoxymethane (10 mg/kg body weight in saline, I.P., 3 times), DSS : Dextran sodium sulfate (2% drinking water for a week).

Table 1. Composition of the diets

Normal Fe Groups	Fe	Fe + LSe	Fe + HSe
Ingredients	g/kg	g/kg	g/kg
Casein	200	200	200
L-Cystine	3	3	3
Sucrose	88.9	88.98	88.5
Cornstarch	397.486	397.486	397.486
Dyetrose	132	132	132
Soybean Oil	70	70	70
t-Butylhydroquinone	0.014	0.014	0.014
Microcrystalline Cellulose	50	50	50
Mineral Mix # 215020 (Rx grade, no Fe Se)	37	37	37
Ferric Citrate P.M 5 mg/g Fe	9	9	9
Na Selenate P.M 1 mg/g Se	0.1	0.02	0.5
Vitamin Mix # 310025	10	10	10
Choline Bitartrate	2.5	2.5	2.5
Total	1000	1000	1000

Carcinogen treatment

Mice were treated three times intraperitoneally with AOM (10 mg/kg body weight) in saline at 0-2nd week of experiment. Additionally, mice received 2% DSS in the drinking water for 7 days at 3rd week and then no further treatment for 9 weeks. The mice in the normal control group were received an injection of saline.

Sample collection

At 12 weeks of experiment, all mice were sacrificed. Before sacrifice, final body weights were measured. After laparotomy, blood was collected by a syringe from the abdominal aorta and immediately transferred into tubes containing K_3-EDTA and serum separator tubes (Vacutainer, Becton Drive Franklin Lakes, NJ, USA). The liver, spleen, kidneys, lung, stomach, small intestine and entire large intestine were harvested. One fifth of liver, spleen and kidneys were washed with saline, blotted dry, weighted and then frozen in liquid nitrogen. A half of the large intestine from cecum to anus was longitudinally opened, flushed with saline, and fixed flat between two pieces of filter paper in 10% neutral buffered formalin. The remaining tissues were fixed in 10% neutral buffered formalin.

Blood analysis

Blood samples in EDTA tubes were used for analysis of complete blood cell count with Abbott CellDyn-3500 (Abbott Laboratories, Chicago, IL, USA).

Histopathological examination

The tissues fixed in 10% neutral buffered formalin were paraffin-embedded, cut to multiple 4-μm sections, and stained with hematoxylin and eosin for histopathological examination under a light microscope (Olympus Tokyo, Japan).

Iron and selenium analysis in liver

For the determination of total iron, samples of liver were analyzed by ICP-AES (Inductively coupled plasma spectrophotometer) (JY 38 Plus, JOBIN-YVON, France). Samples of the frozen material were digested and ashed at 200°C for 4 h using concentrated nitric acid and hydrogen peroxide. For the ICP-AES analysis of iron, the digested sample was diluted with equal amounts of de-ionized water before analysis.

Glutathione peroxidase activity

The GPx activity was measured using a commercial kit (Cayman, MI, USA). This method is based on the principle that GPx can catalyze the action of hydrogen peroxide and glutathione into water and oxidized glutathione. Oxidized glutathione is recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The results of this enzymatic assay are given in units of GPx activity per milligram of protein (nmol/mg protein). The extinction coefficient for NADPH at 340 nm is 0.00622 μM^{-1} cm^{-1}. The protein concentrations were determined according to the method of Lowry (1951), using BSA as a standard.

AC and ACF counts

The colon were removed and flushed with 0.85% NaCl solution and fixed in 10% neutral phosphate buffered formalin. The formalin-fixed colonic tissues were stained in 0.5% methylene blue solution for 30 sec. The total number of ACF and the number of aberrant crypts (AC) in each focus were counted under a microscope (40-100 ×). ACF were identified with the following morphological characteristics: 1) the enlarged and elevated crypts than normal mucosa and 2) increased pericryptal space and irregular lumens. The density of AC/ACF is the number of ACF per square centimeter of mucosa surface.

Tumor incidence

Macroscopically, colonic neoplasms developed with a different tumor incidence for each groups. Tumor incidence was defined as number of mice with tumors/total mice in the group.

TUNEL assay

Levels of apoptosis in distal colon tissue were determined using the TdT-mediated dUTP nick-end labeling (TUNEL) method. 4-μm formalin-fixed, paraffin-embedded tissue sections from the distal colon were processed according to manufacturer's instructions for the ApopTag peroxidase in situ Apoptosis Detection Kit (Intergen Company, Purchase, NY).

Immunohistochemistry of PCNA

The 4-μm formalin-fixed, paraffin-embedded distal colon sections were subjected to deparaffinization and hydration prior to quenching of endogenous peroxidase activity (3% H_2O_2 in methanol for 15 min). An antigen-unmasking step was done by placing the slides in a pressure cooker containing 0.01 M citrate buffer (pH 6.0) for 5 min. The sections were incubated for 60 min with the primary anti-proliferating cell nuclear antigen (PCNA) mouse monoclonal antibody (Santa cruz) was applied for 60 min in a 1:200 dilution. Slides were processed with the ABC reagent from Vectastain Elite (Vector Laboratories) using DAB as the substrate. Sections were counterstained with mayer hematoxylin. Twenty fields, randomly selected from each slide, were analyzed at 100 × magnification.

Immunohistochemistry of β-catenin

The 4-μm formalin-fixed, paraffin-embedded distal colon sections were subjected to deparaffinization and hydration prior to quenching of endogenous peroxidase activity (3% H_2O_2 in methanol for 15 min). An antigen-unmasking step was done by placing the slides in a pressure cooker containing 0.01 M citrate buffer (pH 6.0) for 5 min. The sections were incubated for 60 min with the primary anti-β-catenin rabbit polyclonal antibody (Santa cruz) was applied for 60 min in a 1:500 dilution. Slides were processed with the ABC reagent from Vectastain Elite (Vector Laboratories) using DAB as the substrate. Sections were counterstained with mayer hematoxylin. Twenty fields, randomly selected from each slide, were analyzed at 100 or 200 × magnification.

Statistical analysis

Data were expressed as means ± standard error (SE). Data were analyzed with One-way ANOVA and Tukey HSD using SPSS v 12.0 software. The significant differences were statistically determined at the level of $p < 0.05$ or $p < 0.01$.

Results

Body weights of mice

The body weights of mice induced by AOM followed by DSS slightly decreased (Fig. 4). Such changes in the body weights might be due to the reduction of feed consumption.

Differential blood cell counts

There were no significant changes among normal control group, AOM/DSS alone group and AOM/DSS + selenium deficient diet group in all blood cell counts. However, the mice that were fed a selenium overloaded diet significantly elevated white blood cells and hematocrit compared with normal control group (Table 2).

Selenium concentration in liver

Selenium in the liver was determined using an inductively coupled plasma spectrophotometer. A selenium-overloaded diet was associated with an increase of liver selenium concentrations compared with the AOM/DSS alone group (Fig. 3).

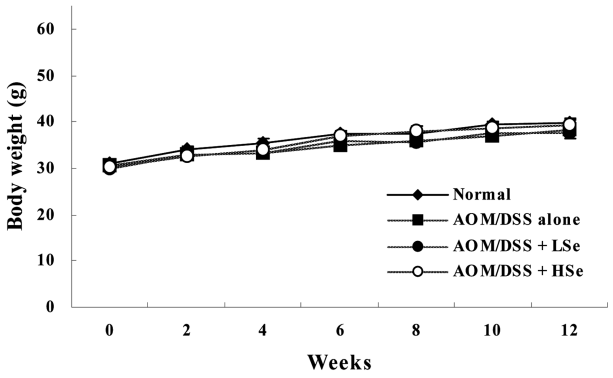

Fig. 2. Change by treatments of AOM/DSS and selenium in the body weights of mice. AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: High selenium diet. Data represented mean ± SE.

Table 2. Differential blood cell counts in mice

	Normal control	AOM / DSS		
		-	LSe	HSe
WBC (thousands)	3.59 ± 0.23	1.95 ± 0.36	2.12 ± 0.51	1.67 ± 0.24*
RBC (millions)	8.68 ± 0.19	7.99 ± 0.10	8.08 ± 0.39	7.98 ± 0.32
HB (g/dl)	12.74 ± 0.27	12.59 ± 0.32	13.12 ± 0.27	12.32 ± 0.42
HCT (%)	45.32 ± 0.70	40.40 ± 0.91	43.00 ± 1.51	40.37 ± 1.04*
MCV (fl)	52.29 ± 0.84	51.79 ± 0.99	51.45 ± 0.87	51.16 ± 0.85
MCH (pg)	16.14 ± 0.33	15.78 ± 0.28	15.12 ± 0.46	15.49 ± 0.28
MCHC (g/dl)	30.88 ± 0.40	30.51 ± 0.53	30.56 ± 0.84	30.31 ± 0.20

WBC: white blood cells, RBC: red blood cells, Hb: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: high selenium diet. Data represented mean ± SE.
*significantly different from normal control ($p < 0.05$).

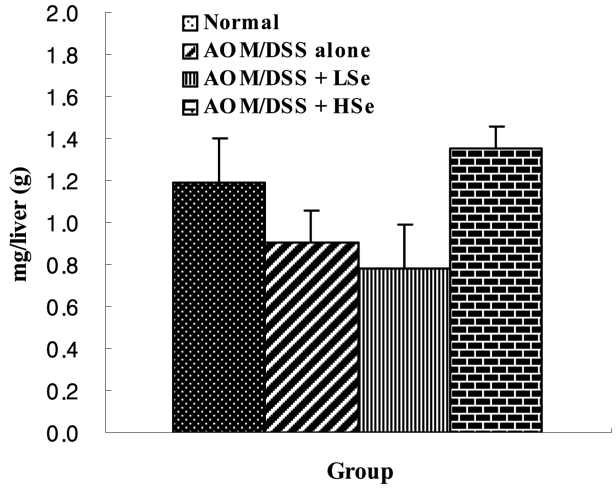

Fig. 3. Hepatic selenium levels in mice. The selenium concentration was determined using an inductively coupled plasma spectrophotometer. Each bar represents the mean value ± SE. AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: high selenium diet.

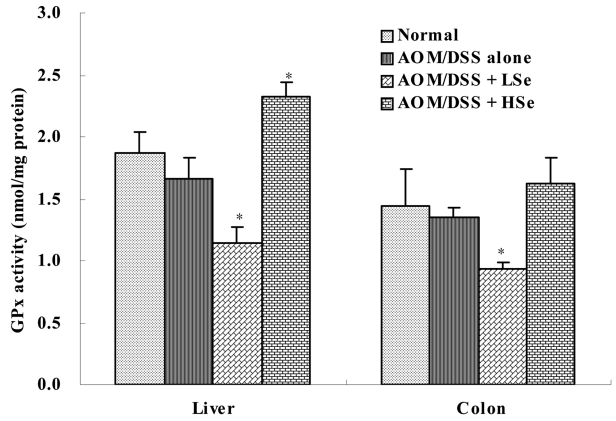

Fig. 4. Effect of selenium on glutathione peroxidase activity in the liver and colon of ICR mice. AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: high selenium diet. Data represented mean ± SE. *Significantly different from AOM/DSS alone ($p < 0.05$).

Glutathione peroxidase activity in liver and colon

As shown in Fig. 4, the GPx levels in the liver and colon in this study were 1.9 ± 0.2 nmol/mg protein and 1.4 ± 0.3 nmol/mg protein for the normal control group, 1.7 ± 0.2 nmol/mg protein and 1.3 ± 0.1 nmol/mg protein for the AOM/DSS alone group, 1.1 ± 0.1 nmol/mg protein and 0.9 ± 0.1 nmol/mg protein for the AOM/DSS + LSe group, and 2.3 ± 0.1 nmol/mg protein and 1.6 ± 0.2 nmol/mg protein for the AOM/DSS+HSe group, respectively. The mice that were fed a selenium-deficient diet showed a significantly decreased activity level of GPx compared with the AOM/DSS alone group in the liver and colon ($p < 0.05$). Similarly, the mice that were fed a selenium-overloaded diet showed an increased activity level of GPx compared with the AOM/DSS alone group in the liver ($p < 0.05$).

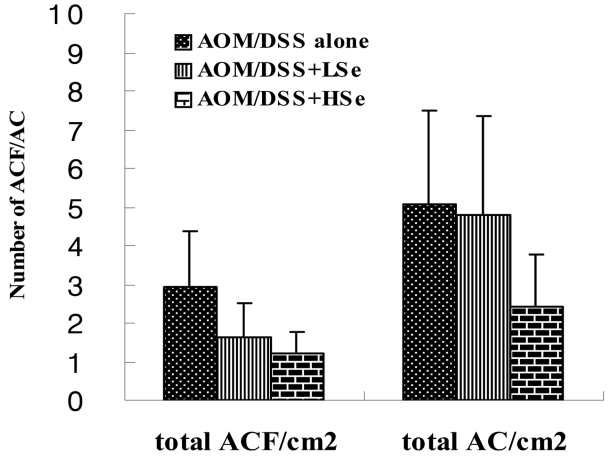

Fig. 5. Effect of selenium on colonic aberrant crypt formation in ICR mice. AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: high selenium diet. Data represented mean ± SE.

Aberrant crypt foci (ACF)

The animals administered with saline (vehicle) showed no evidence of ACF formation in the colon. Treatment with AOM/DSS induced the number of 2.95 ± 2.4 ACF/cm^2, which were composed of a total number of 5.06 ± 4.1 AC/cm^2. The mice that were fed a selenium-deficient diet showed a decrease in the total number of ACF/cm^2 to 1.64 ± 1.5, and the mice that were fed a selenium-overloaded diet showed a decrease in the total number of ACF/cm^2 to 1.22 ± 0.9 and the total number of AC/cm^2 to 2.42 ± 2.3 (Fig. 5).

Tumor incidence

Compared to the mice that were fed a normal selenium diet, the mice that were fed a selenium-deficient diet had a 16% higher in tumor incidence. The percentage of tumor incidence in mice that were fed a selenium-deficient diet was 4% lower than that found in the AOM/DSS alone group (Fig. 6).

Apoptosis in tumor sections

The apoptotic bodies in tumor sections of control and experimental mice were visualized by TUNEL staining and representative micrographs are shown in Fig. 7. The tumors from the mice that were fed a selenium-overloaded diet exhibited a markedly higher count of brown-color apoptotic bodies compared with the AOM/DSS alone group.

Immunohistochemistry of PCNA

The tumor cell proliferation was evaluated by quantification of the cells positive for PCNA, a marker of cell proliferation. The high selenium diet group showed a decrease in the number of proliferated cell staining area compared with the

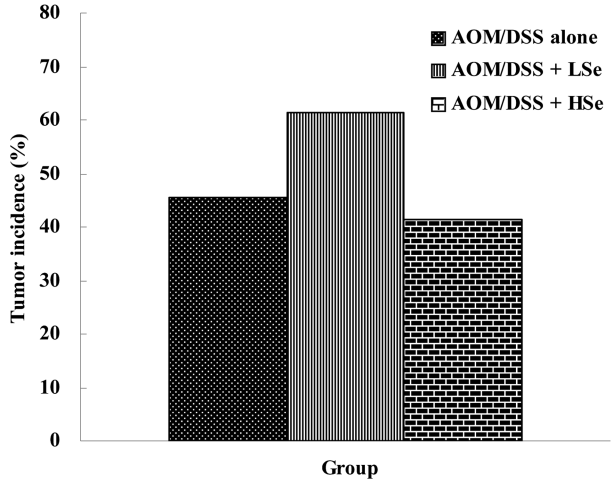

Fig. 6. Effect of selenium on tumor incidence in ICR mice. AOM: azoxymethane, DSS: dextran sodium sulfate, LSe: low selenium diet, HSe: high selenium diet.

AOM/DSS alone group and the other treatment group (Fig. 8).

Immunohistochemistry of β-catenin

The immunoreactivities against β-catenin showed dark brown reaction products with slight variation in AOM/DSS treatment groups. However, the mice that were fed a selenium-overloaded diet group showed a decrease in the β-catenin staining area compared with the AOM/DSS alone group and the other treatment group (Fig. 9).

Discussion

The current study of AOM/DSS-induced colon carcinogenesis in the mouse model elucidated the effects of selenium on colon carcinogenesis induced by AOM and DSS. In the previous study, AOM treatment during or after DSS exposure did not produce a significant number of colonic neoplasm in mice, while DSS exposure after AOM treatment could induce a number of colonic neoplasms[12]. In addition, treatment with the non-genotoxic carcinogen DSS enhances the development of putative precursor lesions (ACF) for colonic adenocarcinoma in rats[26]. Therefore, in the present study, the mice were given a low dose of AOM for initiation three times and a 1-week administration of DSS for induction of colitis. ACF are putative pre-neoplastic lesions that have been detected in human colon resections and in experimental animals treated with chemical carcinogens[4,5]. This characteristic of ACF favors their use as a biomarker for investigating modulation of colon carcinogenesis.

Mice fed a selenium-overloaded diet decreased the formation of ACF/cm^2 and AC/cm^2, compared with the normal selenium group in normal iron status. The percentages of tumor

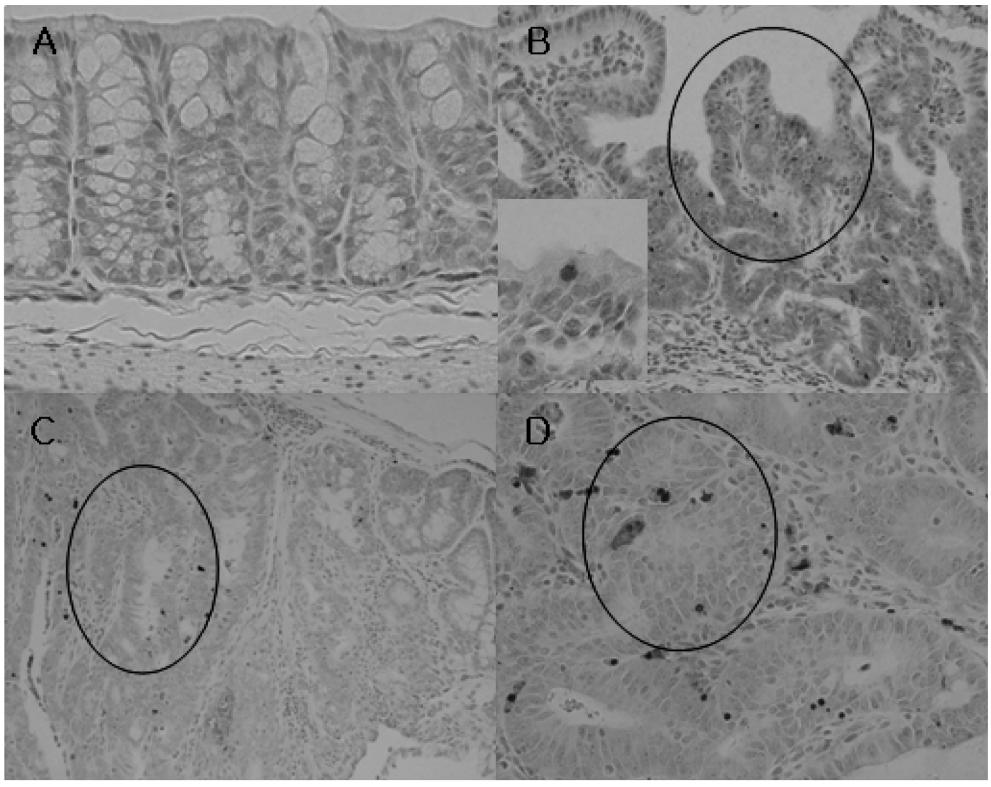

Fig. 7. TUNEL assay for apoptotic nuclei in distal colon sections of mice with normal iron status. Normal control (A) AOM/DSS alone, (B) AOM/DSS + LSe, (C) AOM/DSS + HSe, (D) (× 100 or 200).

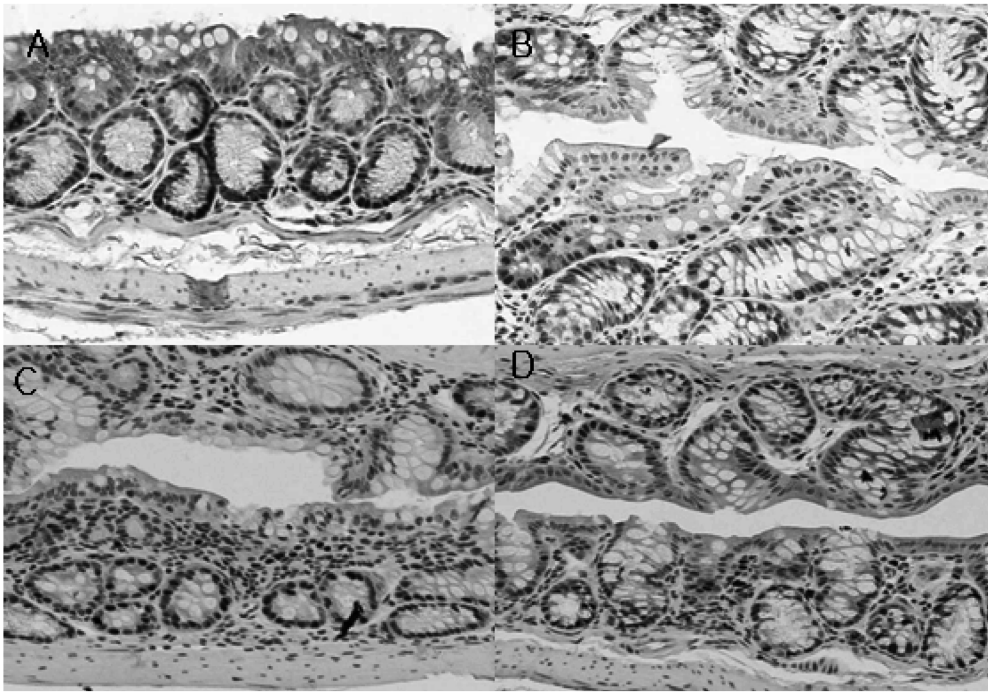

Fig. 8. Immunohistochemistry of PCNA in the colon of normal ICR mice. Normal control (A) AOM/DSS alone, (B) AOM/DSS + LSe, (C) AOM/DSS + HSe, (D) (× 100).

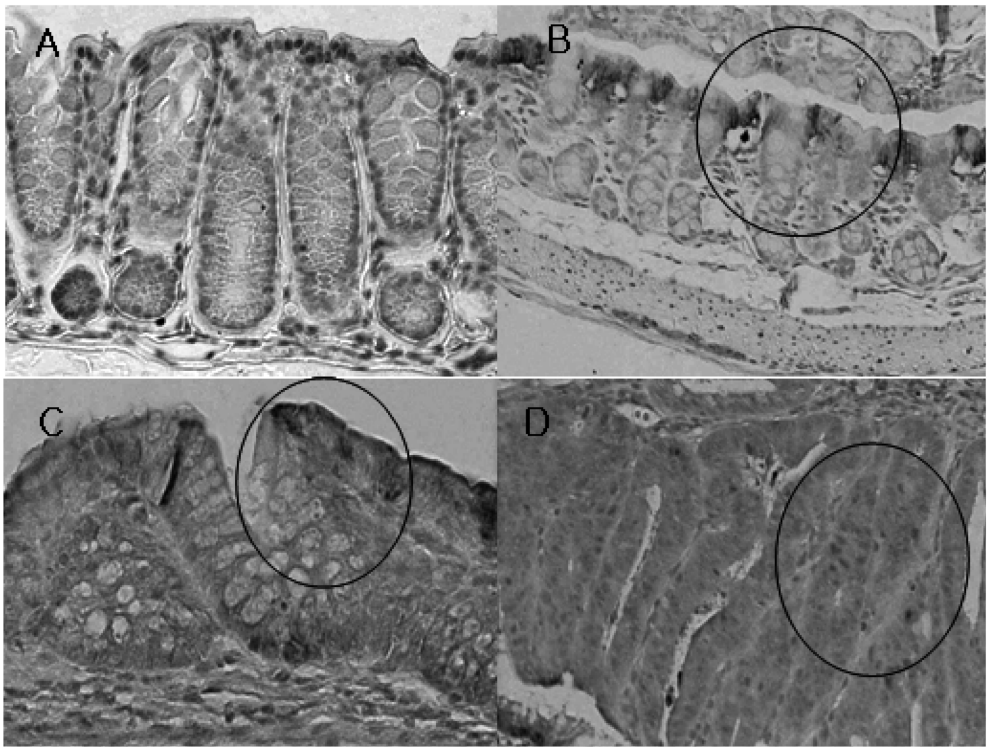

Fig. 9. Immunohistochemistry of β-catenin in the colon of normal ICR mice. Normal control (A) AOM/DSS alone, (B) AOM/DSS + LSe, (C) AOM/DSS + HSe, (D) (× 100 or 200).

incidence in mice fed a selenium-overloaded diet were 4% lower than that found in the normal selenium diet group. On the other hand, tumor incidences in mice fed a selenium-deficient diet were 16% higher than that found in the normal selenium diet group. Clark et al.[27] showed that quite high selenium supplements in individuals with a previous history

of skin cancer led to a reduction in the risk of a number of carcinomas over a 4.5-year period. Similarly, a recent study by Connelly-Frost et al.[28] observed that high selenium was associated with a reduced prevalence of colorectal adenomas. Selenium could reduce the incidence of adenomas through several anticarcinogenic pathways, including the repair and prevention of oxidative damage, alteration of metabolism of carcinogenic agents, regulation of immune response and repair of DNA damage[20,21]. It is also possible that selenium could cause adenomas to regress, shrink or grow more slowly instead of preventing their initial growth. Any of these circumstances could result in an inverse association between selenium and colorectal adenomas[28].

In a previous study, there was no evidence that selenium reduces the colon carcinogenesis in spite of low selenium diet. There are several hypothesis on the mechanism by which selenium reduces colon carcinogenesis. The most likely ones appear to be enhanced apoptosis and increases in DNA repair. It is proposed that selenium does not reduce carcinogenesis by a single mechanism, but instead by multiple ones[29].

As the results showed, the activity of GPx in the liver and colon was decreased in the selenium-deficient diet group while it was increased in the selenium-overloaded diet. Like preceding report, the results showed that the acitivity of GPx, which is one of the antioxidants, is enable to increase or decrease based on the level of the selenium diet intake. In this research, we performed TUNEL stain and immunohistochemistry of PCNA and β-catenin on the mucous membrane tissue of colon in order to confirm the additional results. On TUNEL stain, apoptotic positive cells were increased or decreased in the selenium-overloaded diet group and selenium-deficient diet group, respectively. It is thought to be that the apoptotic cells were increased or decreased due to the changes of the activity of anitioxidant GPx based on the selenium intake. On the immunohistochemistry staining of PCNA among the selenium-overloaded diet group, the PCNA staining area was inhibited in the normal tissue and abnormal tissue which was considered to be a tumor. It is thought to be that the proliferating cells were relatively decreased due to the increased GPx activity as a result of the selenium intake. In addition, the β-catenin protein level, which is an early carcinogenesis biomarker, was increased in some selenium-deficient diet group. On the contrary, the β-catenin protein level was decreased in the selenium-overloaded diet group, which is thought to be related to the increased GPx activity.

In summary, a high selenium diet reduce colon carcinogenesis. These findings indicate that dietary selenium might exert a modulating effect on colon cancer by inhibiting the development of ACF/AC and colon tumor formation in the ICR mice.

요 약

본 연구에서는 azoxymethane (AOM)과 dextran sodium sulfate (DSS)로 유도된 대장 발암과정에 대한 셀레늄의 방어 효과를 조사하였다. 셀레늄 결핍(0.02 ppm Se), 정상(0.1 ppm Se), 과다(0.5 ppm Se)사료를 12주간 식이로 급여하여 혈액검사와 대장암 발생의 초기단계인 aberrant crypt foci (ACF)수를 측정했으며, 암 발생율을 조사하였다. ICP-AES를 사용하여 간의 셀레늄 농도를 측정하였으며, 또한 셀레늄포함 항산화효소인 glutathione peroxidase (GPx) 활성을 알아보았다. 또한 TUNEL assay와 PCNA, β-catenin에 대한 면역조직 염색을 수행하였다. ACF 수 및 종양 발생률에 있어서, 셀레늄과다사료를 급여한 군이 정상셀레늄사료를 급여한 군보다 낮았으며, 셀레늄결핍사료를 급여한 군은 오히려 ACF 수 및 종양 발생률이 높았다. GPx 활성은 셀레늄의 섭취가 과다한 군에서 높게 나타났으며, 이 때, TUNEL에서 apoptotic positive cell이 증가하는 것을 확인했다. 또한 셀레늄의 섭취가 과다한 군에서 PCNA와 β-catenin의 발현이 감소됨을 볼 수 있었다. 본 마우스 모델실험에서 셀레늄은 여러 기전에 의해 대장암 발생을 억제할 수 있을 것으로 사료된다.

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