Introduction for inflammation and cancer

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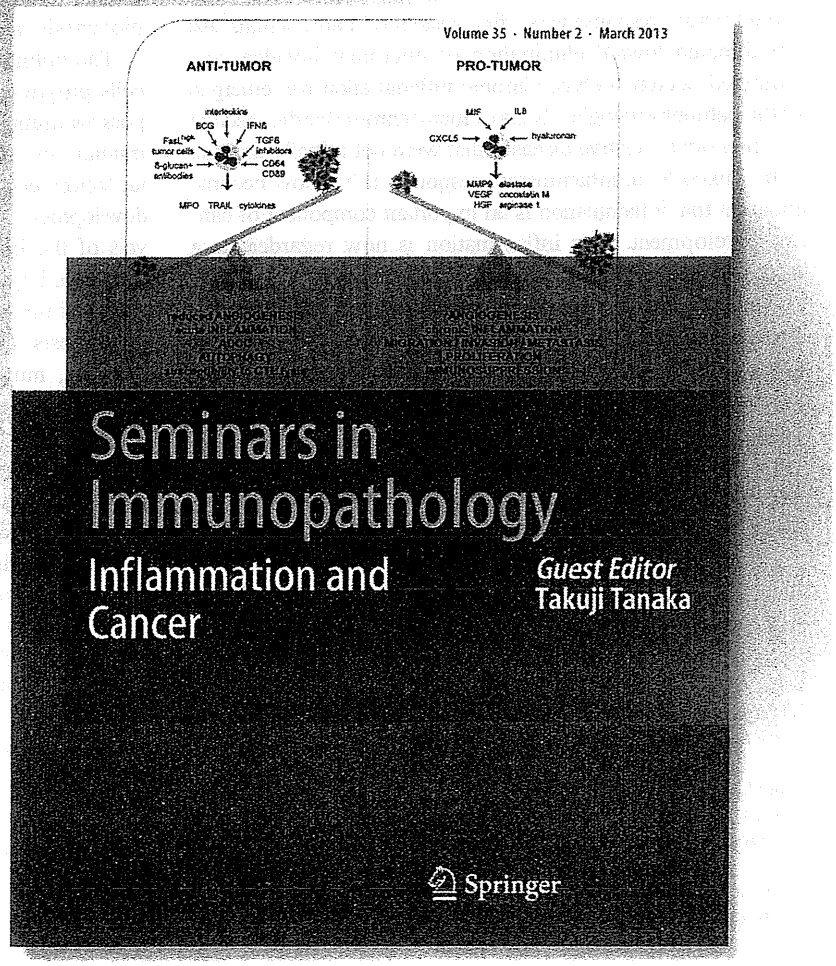

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INTRODUCTION

Introduction for inflammation and cancer

Takuji Tanaka

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How inflammation protects or injures tissues of the human body is quite an important issue, particularly in cancer development. Inflammation also affects the immune system or immune surveillance. We now know the importance of inflammation and the innate/adaptive immune system in tumor development, promotion, progression, and metastasis.

Inflammation has two faces in human tissues. The acute inflammatory response is the first system of alarm signals that are directed toward elimination of microbial invaders and wounded necrotic debris. Chronic inflammation has emerged as the pathophysiological basis of many chronic cardiovascular and neurodegenerative diseases that were not initially thought to be linked to an inflammatory response. It has now become apparent that inflammation is an important component of cancer development. This inflammation is now regarded as a "secret killer" of chronic diseases, such as cancer.

The concept of a relationship between inflammation and carcinogenesis is not new. Based on the presence of leukocytes within cancer tissue, the founder of cellular pathology, Dr. Rudolph Virchow, speculated that there was an association between inflammation and cancer development in 1863. In line with his notion, epidemiological data indicate that inflammation serves as a potential risk factor for the development of cancer in different tissues. It is generally accepted that up to 25 % of human malignancies are related to chronic inflammation with or without (viral, bacterial, or parasitic) infections. Evidence shows that inflammatory stimuli caused by inflammation increase the risk of cancer, promote tumor progression, and support metastatic spread. Thus, inflammatory cells and cytokines act as tumor promoters that affect cell survival, proliferation, invasion, angiogenesis, and resistance of chemotherapy.

The immune system always acts to prevent preneoplastic cells progressing to cancer. On the other hand, cancer cells possess immunosuppressive properties such that they escape immune surveillance. In addition, we know that environmental factors decrease the immune function, resulting in cancer development. These observations together indicate that analysis of the immune function could contribute to the early detection, prevention, and treatment of cancer.

Based on the types of inflammation associated with carcinogenesis, the tumor microenvironment can include cancer cells, innate immune cells (macrophages, neutrophils, mast cells, dendritic cells, natural killer cells, and myeloid-derived suppressor cells), adaptive immune cells (T and B lymphocytes), and stromal cells (fibroblasts, vessel endothelial cells, and other mesenchymal cells). To maintain the growth of cancer, these cells communicate with each other directly or indirectly by producing cytokines and chemokines in autocrine and/or paracrine manners.

This special issue entitled "Inflammation and Cancer" is mainly focused on this topic but also touches on the prevention of inflammation-related cancers. Several world-class experts from diverse fields and institutions have written review articles for this introductory special issue of the journal. Each of the reviews is presented as an introduction by experts who are involved in cutting-edge research in their area of expertise. Dr. Sven Brandau et al. describe the pro-tumor and anti-tumor functions of neutrophil granulocytes in tumorigenesis. They propose a model where homeostatic chronic recruitment and activation of neutrophils result in

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Mast cells and inflammation-associated colorectal carcinogenesis

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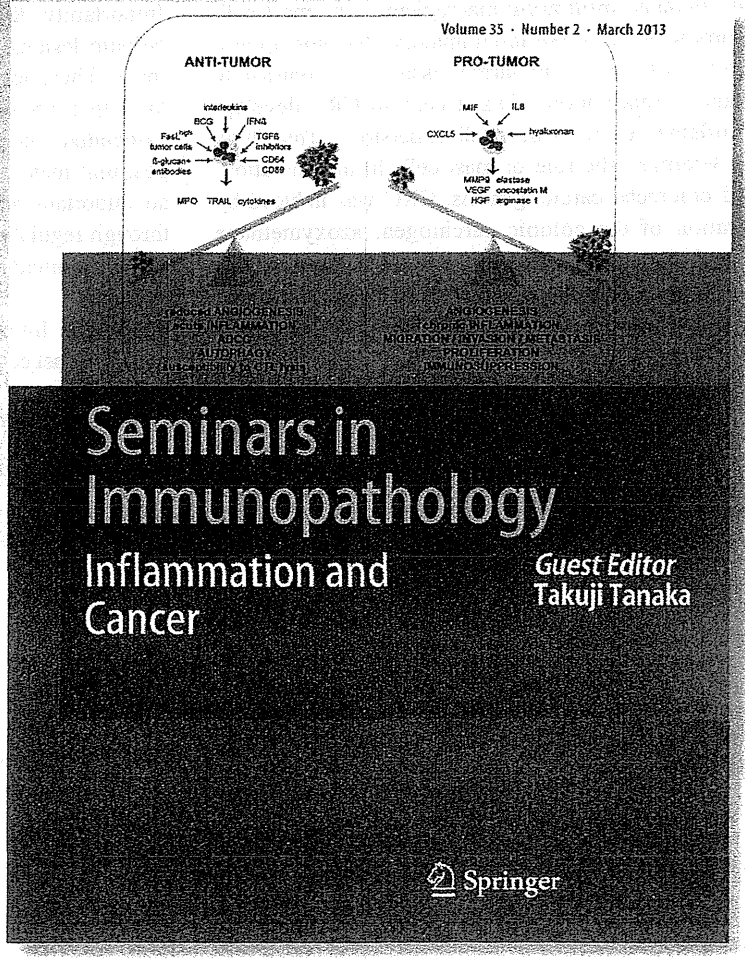

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REVIEW

Mast cells and inflammation-associated colorectal carcinogenesis

Takuji Tanaka · Hideki Ishikawa

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Abstract Close association between chronic inflammation and cancer has been recently highlighted. Indeed, inflammatory bowel disease (IBD) has been strongly linked with an increased risk of development of colorectal cancer (CRC). Inflammatory cell-produced inflammatory mediators, such as proinflammatory cytokines and inducible enzymes, contribute to this association. In an inflammatory microenvironment, infiltrating macrophages and mast cells mediate production of these inflammatory mediators to promote growth of tumors in target tissues. In contrast to macrophages, contribution of mast cells to CRC development in inflamed colon is not well understood. This study aimed to determine the role of mast cells in inflammation-associated colorectal carcinogenesis. CRC was induced by administration of the colonic carcinogen, azoxymethane (AOM), and the tumor promoter dextran sodium sulfate (DSS) in male mast cell-deficient WBBF$_1$-*kit*$^{W/W\text{-}v}$ (W/W^v) and mast cell-normal WBB6F$_1$-+/+(WT) mice. At week 12, the W/W^v mice had markedly lower inflammation scores in the colon when compared with WT mice. The mRNA levels of colonic proinflammatory cytokines and inducible enzymes were also decreased in W/W^V mice at weeks 12 and 20, when compared with WT counterparts. Colorectal tumors, including CRC, were identified by histopathological analysis performed 20 weeks thereafter. Importantly, there were less neoplastic and preneoplastic colonic lesions in the W/W^v mice compared with the WT mice. Thus, for the first time, our study shows that mice lacking mast cells are less susceptible to inflammation-associated colorectal carcinogenesis. Our findings also suggest that mast cells and their selected cytokines could play an important role in inflammation-mediated tumorigenesis through regulation of proinflammatory cytokines and inducible inflammatory enzymes.

Keywords Inflammation and cancer · IBD · Colorectal cancer · Mast cells · WBBF$_1$-*kit*$^{W/W\text{-}v}$ mice · Cytokines

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Introduction

The association between inflammation and cancer was first perceived in the 19th century and is now accepted as an enabling characteristic of cancer [1–5]. One consistent feature of the tumor microenvironment is smoldering inflammation. Chronic and persistent inflammation increases risk of cancer development in several tissues. Approximately 25 % of cancers are associated with chronic inflammation sustained by infections (e.g., hepatitis, gastritis, and condyloma acuminatum) or inflammatory conditions of diverse origin, including inflammatory bowel disease (IBD) and prostatitis [1–5]. Inflammation and cancer are associated by two pathways: extrinsic and intrinsic. The former pathway causes smoldering inflammatory responses, and the latter is driven by inflammation-associated programs

purchased from MP Biomedicals (Aurora, OH, USA). DSS for induction of colitis was dissolved in water to a concentration of 2 % (w/v).

Animal treatments

A total of 44 male mice of each genotype were used for the experiment. Twenty mice of each genotype were given a single intraperitoneal (i.p.) injection of AOM (10 mg/kg body weight diluted in saline). Seven days after administration of AOM, mice were exposed to drinking water containing 2 % DSS for a period of 7 days. Other experimental groups of each genotype included AOM alone (n=8), DSS alone (n=8), and untreated (n=8). At week 12, four mice of each genotype, which were treated with AOM and DSS were euthanized by CO_2 asphyxiation for analysis of mRNA expression. Colons (distalmost 1 cm) were gently removed from the underlying muscular layer with a glass microscope slide [30] for analysis of mRNA expression of the proinflammatory cytokines including nuclear factor kappaB2 (NF-κB2), TNF-α, interleukin (IL)-1β, and IL-6 as well as inducible inflammatory enzymes, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS). Similarly, five mice of each genotype that received AOM and DSS and four mice each from other treatment groups (AOM alone, DSS alone, and untreated) were euthanized and colons were used for the mRNA expression analysis of the aforementioned cytokines, with the exception of NF-κB2 and inducible inflammatory enzymes at week 20 (18 weeks after the cessation of DSS exposure). At week 20, the remaining mice were also euthanized by CO_2 asphyxiation and their colons were removed for histopathological examination after hematoxylin and eosin (H&E) staining. At sacrifice, colons were removed and washed in phosphate-buffered saline. They were opened longitudinally, and feces were removed. The large intestines were then macroscopically inspected and the volume of tumors, if present, was measured. Tumor volume was calculated using two formulas: formula 1 ($a \times b^2/2$) and formula 2 ($a \times b^2 \times \pi/6$), where a and b represent the long and short diameters, respectively. For histopathological and immunohistochemical analyses, the opened colon was split longitudinally, fixed in 10 % neutral-buffered formalin, and embedded in paraffin. As did at week 12, the whole colonic mucosa without tumors was scraped with a glass microscope slide for analysis of mRNA expression of the proinflammatory cytokines (TFN-α, IL-1β, and IL-6) and inducible inflammatory enzymes (COX-2 and iNOS). Scraped samples of colonic mucosa that were collected at weeks 12 and 20 for mRNA expression analysis were placed in cryogenic vials, fast-frozen in liquid nitrogen, and stored at −80 °C until analyzed. All animal experiments were performed in accordance with protocols approved by the Animal Care and Use Committee of the Institute, TCI-CaRP.

Histopathological and immunohistochemical analysis

Four-micrometer sections of the colon paraffin tissue blocks were stained with H&E. At week 12, inflammation in the colon of the AOM/DSS group of each genotype (n=4) was graded according to the following morphological criteria [31]: Grade 0, normal appearance; Grade 1, shortening and loss of the basal one-third of the actual crypts with mild inflammation in the mucosa; Grade 2, loss of the basal two-thirds of the crypts with moderate inflammation in the mucosa; Grade 3, loss of the entire crypts with severe inflammation in the mucosa and submucosa, but with retainment of the surface epithelium; and Grade 4, presence of mucosal ulcer with severe inflammation (neutrophils, lymphocytes, macrophages, and plasma cell infiltration) in the mucosa, submucosa, muscularis propria, and/or subserosa. The scoring was made on the distal 1/2 colon with or without proliferative lesions and expressed as mean average score per mouse. At week 20, large bowel lesions were evaluated for the presence of mucosal ulcers, preneoplasms (dysplastic crypts), adenoma, and adenocarcinoma [14, 32]. For assessment of the presence or absence of mast cells, histology sections from the colons of both mouse genotypes were immunohistochemically stained with 500-fold diluted anti-tryptase antibody (AA1 clone, Dako Japan, Kyoto, Japan), as described previously [33].

Quantitative RT-PCR analysis

Total RNA was extracted from scraped colonic mucosa homogenates using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's protocol. cDNA was then synthesized from total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Japan Ltd., Tokyo, Japan). Quantitative real-time PCR analysis of individual cDNAs was performed with the ABI Prism 7500 (Applied Biosystems Japan Ltd., Tokyo, Japan) using TaqMan Gene Expression Assays (Applied Biosystems Japan Ltd., Tokyo, Japan; *Nf-kappaB2*, Mm00479807_m1; *Tnf-α*, Mm00443258_m1; *Il-1β*, Mm00434228_m1; *Il-6*, Mm00446190_m1; *inos*, Mm00440485_m1; and *β-actin*, Mm00607939_s1). PCR cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The expression level of each gene was normalized to the β-actin expression level using the standard curve method.

Statistical analysis

All measurements were statistically analyzed using the unpaired t test (two-tailed), Tukey multiple comparison post

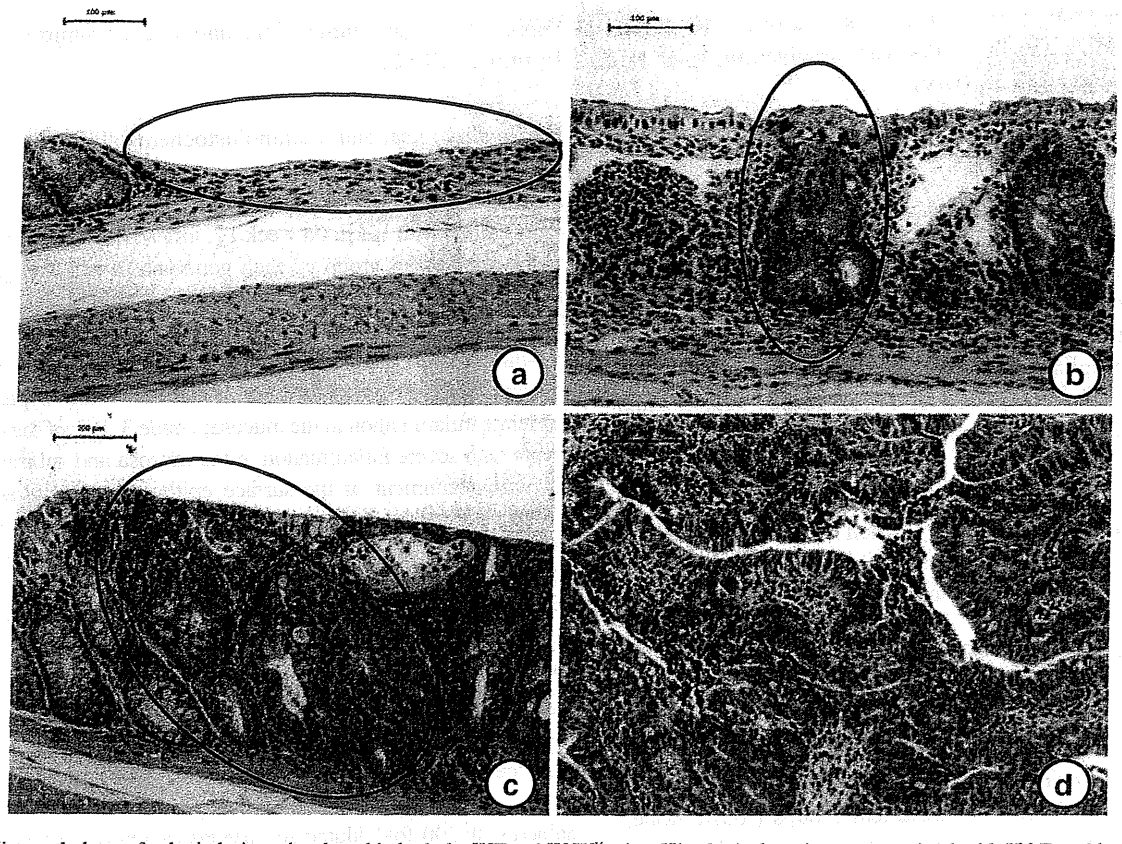

Fig. 3 Histopathology of colonic lesions developed in both the WT and W/W^{v} mice. Histological sections were stained with H&E and key feature are indicated: mucosal ulcer (**a**, *circled*), dysplastic crypts (**b**, *circled*), tubular adenoma (**c**, *circled*), and tubular adenocarcinoma (**d**). Bars = 100 μm

adenoma, and adenocarcinoma of the WT mice that received AOM and DSS (Fig. 6a, c, and e), while none or few were present in the W/W^{V} mice treated with AOM and DSS (Fig. 6b, d, and f). Thus, these data reiterate that mast cells are recruited during colitis and CRC in mice. In addition, these data validate the mast cell-deficient W/W^{V} mouse model.

mRNA expression of proinflammatory cytokines (NF-κB2, TNF-α, IL-1β, and IL-6) and inducible inflammatory enzymes (COX-2 and iNOS)

Next, the mRNA expression profiles of key cytokines and inducible inflammatory enzymes were analyzed using colons from WT and W/W^{V} mice after induction of CRC. The mRNA expression of the cytokines NF-κB2, TNFα, IL-1β, and IL-6, as well as the inducible inflammatory enzymes, COX-2 and iNOS, were examined in the colonic mucosa of W/W^{V} and WT mice at weeks 12 and 20, respectively. At week 12, expression of all the cytokines and inducible inflammatory enzymes examined were significantly lower in the colonic mucosa of the W/W^{V} mice that received AOM and DSS than that of similarly treated WT mice. At week 20, the expression levels of the proinflammatory cytokines (TNFα, IL-1β, IL-6, and COX-2) and inducible inflammatory enzymes (COX-2 and iNOS) in the colonic mucosa of the W/W^{V} mice that received AOM and/or DSS remained lower than that of the WT mice, but the differences between the two genotypes were not statistically significant. These findings suggest that mast cell deficiency alters the colonic inflammatory profile in our colitis-related CRC mouse model.

Discussion

In this study, we investigated AOM/DSS-induced colorectal carcinogenesis in W/W^{V} and WT mice to determine a possible role for mast cells in inflammation-associated colorectal carcinogenesis. Our findings clearly indicated that W/W^{V} mice that lack mast cells were less susceptible to AOM/DSS-induced colorectal carcinogenesis when compared with similarly treated WT mice. Also, two genotypes have different degrees of inflammation in the colon (Fig. 2) and their differences in tumor volume and multiplicity might be due to this factor. The results, thus, suggest the importance of inflammatory stimuli in the AOM and DSS models.

In the current study, tryptase-positive mast cells were not identified in the colonic mucosa of the W/W^{V} mice by immunohistochemical staining, suggesting that this

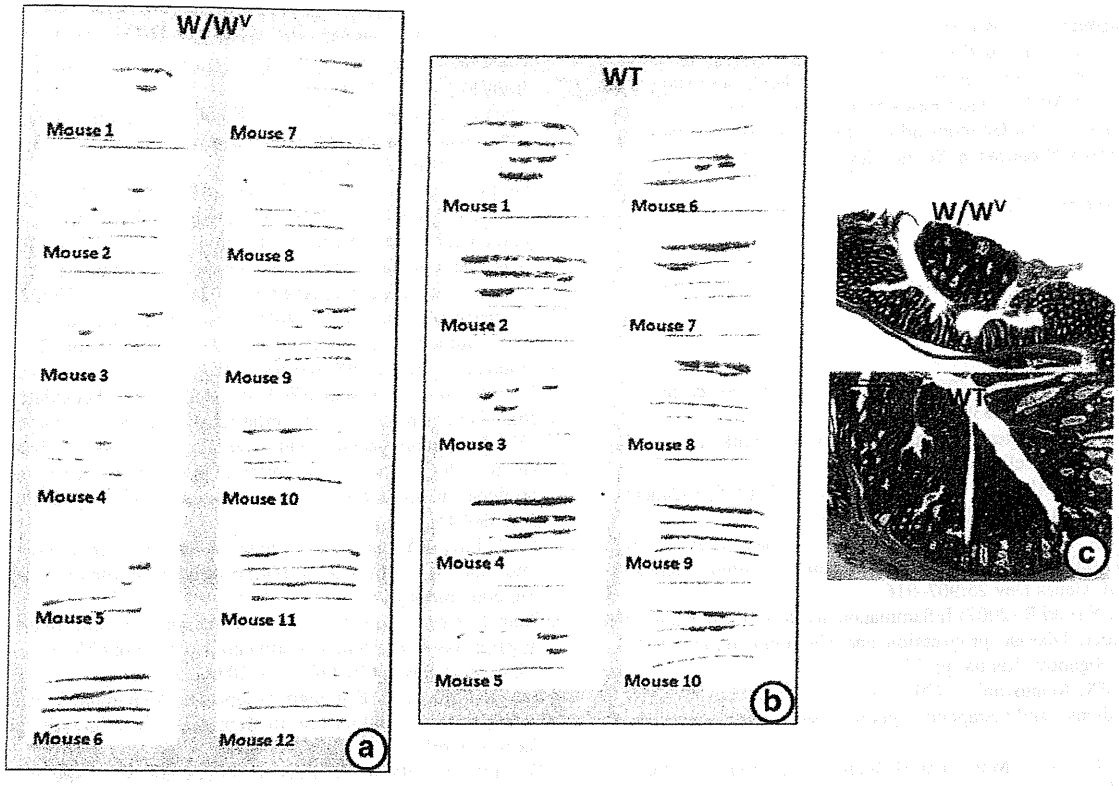

Fig. 5 Colonic tumors on the histologic sections from the W/W^V (**a**) and the WT mice (**b**). **c** Representative tumor size difference between a WT and W/W^V mouse in the histologic sections. A total of 31 and 56 colonic tumors developed in the W/W^V and WT mice, respectively. The number and size of tumors developed in the W/W^V (n=28) and WT mice (n=50) were quantified. A Tukey multiple comparison post test and an unpaired t test (two-tailed) were performed to examine differences in the tumor number and size between the W/W^V and WT mice, respectively

PGE_2 are also released at sites of inflammation during chronic inflammation [41]. Nitrosative stress caused by iNOS-derived NO is strongly associated with IBD progression and contributes to the pathogenesis of human IBD, experimental colitis [48], and colitis-associated colon tumorigenesis [49]. COX-2 is another factor involved in IBD [50] and colitis-related colon carcinogenesis [49] by acting through the synthesis of PGE_2.

In carcinogenesis, a role for mast cells in cancer development and progression has been suggested [20, 51, 52]. It has been reported that mast cells act to promote the "angiogenic switch" during tumor growth [53]. Mast cell infiltration within or around tumors, such as CRC [54], is associated with poor prognosis, although the association depends on the type of cancer [51]. In the W/W^V mice, tumor growth of subcutaneously injected B16–BL6 melanoma cells has been shown to decrease at early stages but was recovered after reconstitution of the mast cell population [55]. Mast cells are reported to be causatives in colonic adenoma formation, the early step of colorectal carcinogenesis, in humans [22]. In this study, the expression of TNF-α, a major product of mast cells [56] that acts at the apex of proinflammatory pathways in IBD [57] and promotes CRC [58], was decreased in the colon of mast cell-negative W/W^V mice compared with WT mice after induction and promotion of CRC. TNF-α was most significantly decreased when expression was examined 12 weeks after initiation with AOM. At this same time point, expression of NF-κB2 in the colonic mucosa of W/W^V mice was lower than that of the WT mice. NF-κB is considered to play a pivotal role in immune and inflammatory responses through the regulation of genes encoding proinflammatory cytokines and inducible enzymes, such as COX-2 and iNOS. Thus, our findings suggest that NF-κB, as well as cytokines and inducible enzymes, are rationale targets for either prevention or treatment of colitis and colitis-related colon carcinogenesis [59].

In colitis-associated CRC, DNA injury is induced by production of free radicals and the iNOS system in the colonic mucosa with persistent inflammation, followed by *p53* mutation and development of a precancerous lesion, dysplasia. Dysplasia progression to invasive CRC is mediated by COX-2, iNOS, and several cytokines produced by infiltrating inflammatory cells and a result of the accumulation of genetic abnormalities, such as loss of the *DCC* gene [8]. In this study, we observed the expression of COX-2 and

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Conflict of interest None.

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Organomagnesium suppresses inflammation-associated colon carcinogenesis in male Crj: CD-1 mice

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Magnesium (Mg) deficiency increases genomic instability and Mg intake has been reported to be inversely associated with a risk of colorectal cancer (CRC). This study was designed to determine whether organo-Mg in drinking water suppresses inflammation-associated colon carcinogenesis in mice. Male Crj: CD-1 mice were initiated with a single i.p. injection of azoxymethane (AOM, 10 mg/kg body weight) and followed by a 1 week exposure to dextran sulfate sodium (DSS, 1.5%, w/v) in drinking water to induce colonic neoplasms. They were then given the drinking water containing 7, 35 or 175 p.p.m. organo-Mg for 13 weeks. The chemopreventive efficacy of organo-Mg was determined 16 weeks after the AOM exposure. Administration with organo-Mg at all doses caused a significant inhibition of CRC development ($P < 0.01$ and $P < 0.001$). Especially, the highest dose of organo-Mg significantly suppressed the occurrence of all the colonic pathological lesions (mucosal ulcer, dysplasia, adenoma and adenocarcinoma). Organo-Mg also significantly reduced the number of mitoses/anaphase bridging, as well as proliferation of CRC. Additionally, at week 4, organo-Mg lowered the messenger RNA expression of certain proinflammatory cytokines, such as interleukin-1β, interleukin-6, interferon-γ and inducible nitric oxide synthase in the lesion-free colorectal mucosa at week 4 but increased the Nrf-2 messenger RNA expression. Our findings that organo-Mg inhibits inflammation-related mouse colon carcinogenesis by modulating the proliferative activities and chromosomal instability of CRC and suppressing colonic inflammation may suggest potential use of organo-Mg for clinical chemoprevention trials of CRC in the inflamed colon.

Introduction

Cancer incidence in the developed countries has increased throughout this century and has already been the leading cause of death in some Western countries (1,2). Despite great advances in the integration of therapies for malignant epithelial malignancies, the 5-year survival rate for individuals with malignancies is still low. There has been a marked increase in the understanding of cell and molecular mechanisms underlying a variety of carcinogenic processes. However, therapeutic options for advanced neoplastic disease remain limited. This lack of treatment alternatives may be due to the large number of genetic and molecular alterations associated with advanced malignancies that contribute to the maintenance of neoplastic progression. Colorectal cancer (CRC) is the third-most common malignancy and the fourth-most common cause of cancer mortality worldwide (3). The chemopreventive approach to inhibit cancer development and progression is highly attractive. Practical limitations may exist with respect to developing novel and effective chemopreventive agents through the use of appropriate animal models for preclinical evaluation of candidate chemopreventive agents.

Magnesium (Mg) is an essential mineral rich in wheat germ, green vegetables, legumes, algae, nuts and seeds, which acts as a cofactor in enzymatic reactions in the human body. Meat, fruit and dairy products have moderate Mg content, whereas refined foods are poor sources of Mg. A large number of studies indicate that a higher consumption of Mg may favorably affect a cluster of metabolic and inflammatory disorders including insulin resistance (4), hypertension (5), dyslipidemia (6), diabetes mellitus (7), metabolic syndrome (6) and cardiovascular disease (5). Epidemiological studies have indicated an inverse association between dietary intake of Mg and incidence of certain types of cancer, including CRC (8,9). We previously reported that magnesium hydroxide in diet significantly suppressed colon carcinogenesis induced by azoxymetheane (AOM) in rats (10) by modulating cell proliferation activity of cryptal cells that was initiated with colonic carcinogens (11).

Patients with two major types of inflammatory bowel disease, ulcerative colitis (UC) and Crohn's disease, are at an increased risk for the development of CRC (12). Unlike sporadic CRC, CRC in UC patients arises from a focal or multifocal dysplastic mucosa in areas of inflammation (12). Chromosomal instability is frequently observed in chronic inflammatory conditions including UC by estimating aneuploidy (13,14). Inflammatory bowel disease-related CRC has also high rate of chromosomal instability when compared with sporadic CRC (15,16). Low Mg promotes oxidative stress and inflammation (17), which generate genetic instability and increases the risk of mutations (18). Inflammation is involved not only in the early stages of tumorigenesis but also in the late events since inflammatory mediators promote invasion and metastasis (18). Tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6 were induced under Mg deprivation (17).

The current study was designed to explore the possible cancer chemopreventive efficacy of Mg. We investigated the effects of Mg in drinking water on large bowel oncogenesis using an AOM/dextran sodium sulfate (DSS)–treated mouse model, which is a useful animal model to study chemoprevention in inflammation-related colon carcinogenesis (19,20). To understand the mechanism(s) by which organo-Mg modify AOM/DSS-induced colon carcinogenesis, expressions of inflammatory enzymes, such as COX-2, inducible nitric oxide synthase (iNOS) and inflammatory cytokines, such as TNF-α, IL-1β, IL-6 and interferon (IFN)-γ in the non-lesional colonic mucosa were examined. Since nuclear factor erythroid 2–related factor 2 (Nrf2), a transcriptional regulator of oxidant responses, expression and activation, plays a critical role in protecting colitis-associated CRC (21–23), mRNA expression of Nrf2 was assessed in colon mucosa. In addition, we determined whether organo-Mg in drinking water affects the chromosomal instability of adenocarcinoma cells by counting the number of anaphase-bridging formations (24,25). Effects of organo-Mg on growth of human colorectal adenocarcinoma cell line, DLD-1, were also evaluated.

Abbreviations: ABI, anaphase bridging index; AOM, azoxymetheane; CIN, chromosomal instability; CRC, colorectal cancer; DSS, dextran sulfate sodium; IL, interleukin; INF, interferon; iNOS, inducible nitric oxide synthase; Mg, magnesium; MCM2, minichromosome maintenance protein 2; Nrf2, nuclear factor erythroid 2–related factor 2; TNF, tumor necrosis factor; UC, ulcerative colitis.

Table I. Body weights and intakes of drinking water

Group no.	Treatment	Body weight (g)		Water consumption (ml/mouse/day)
		Initial (No. of mice)	Final (No. of mice)	
1	AOM/DSS	24.9±1.0[a](21)	43.8±3.1(16)	7.40±0.98
2	AOM/DSS/7 p.p.m. organo-Mg	24.8±1.0(20)	47.6±5.8(15)	7.76±0.57
3	AOM/DSS/35 p.p.m. organo-Mg	25.0±0.9(21)	47.5±4.2(16)	7.75±1.02
4	AOM/DSS/175 p.p.m. organo-Mg	24.8±1.1(20)	46.9±4.6(15)	7.05±0.60
5	175 p.p.m. organo-Mg	24.1±0.5(11)	44.3±4.1(6)	6.83±0.60
6	None	24.9±0.8(12)	45.9±3.4(7)	6.64±1.06

[a]Mean ± SD.

during the study. Food consumption (grams/day/mouse) did not differ significantly among the groups (data not shown). Also, the mean daily intake of drinking water with or without DSS did not significantly differ among the groups (Table I). The mean body weight at the termination (week 20) was not different among the groups (Table I). The mean colon length of group 1 was slightly shorter than that of other groups (data not shown) but it was not statistically significant.

Incidence and multiplicity of colonic lesions

Macroscopic colonic lesions, including tumors and small ulcerations, were seen in the mice in groups 1 through 4 (Figure 1). The mice of groups 5 and 6 did not develop colonic tumors (Figure 1).

Microscopic examinations revealed various pathologic colonic lesions in mice belonging to groups 1–4. The lesions included mucosal ulcers (Figure 2A), low- and high-grade dysplastic crypts (Figurer 2B), tubular adenomas (Figure 2C) and tubular adenocarcinomas with invasion (Figure 2D). Table II summarizes the microscopic data on the incidence and multiplicity of colonic lesions. The mean numbers of mucosal ulcers in groups 3 ($P < 0.05$) and 4 ($P < 0.01$) were also significantly smaller than that of group 1. Similarly, the mean numbers of high-grade dysplastic crypts in groups 3 ($P < 0.05$) and 4 ($P < 0.01$) were significantly lower than that of group 1. In additon, organo-Mg exposure to mice at 175 p.p.m. in the drinking water significantly diminished the incidences of colorectal adenoma and adenocarcinoma ($P < 0.01$ for both the lesions). The administration of 7 p.p.m. organo-Mg (group 2) significantly reduced the multiplicity ($P < 0.01$) of adenocarcinomas and the number of total tumors (adenoma + adenocarcinoma, $P < 0.01$) when compared with group 1. Drinking with 35 p.p.m. organo-Mg (group 3) also significantly lowered the numbers of adenocarcinomas and total tumors when compared with group 1 ($P < 0.01$ for each comparison). Furthermore, intake of 175 p.p.m. organo-Mg (group 4) significantly lowered the numbers of adenomas, adenocarcinomas and total tumors ($P < 0.001$ for each comparison) when compared with group 1.

Scores of inflammation in the colorectum

As illustrated in Figure 3, AOM and DSS treatment induced colitis with an inflammation score 2.06±0.93. Administration with organo-Mg in drinking water significantly lowered the score of inflammation in the colorectum at 7 p.p.m. ($P < 0.05$), 35 p.p.m. ($P < 0.001$) and 175 p.p.m. ($P < 0.001$). The inflammation score of the organo-Mg (175 p.p.m.) alone group was comparable with that of the untreated group.

MCM2-positive indices of adenocarcinomas

The data on the proliferative kinetics in the colonic adenocarcinomas by estimating the MCM2-positive indices are shown in Figure 4. The MCM2-positive indices for colonic adenocarcinomas in groups 2 (48.9±20.0, $P < 0.001$), 3 (40.5±16.4, $P < 0.001$) and 4 (28.6±8.4, $P < 0.001$) were significantly lower than in group 1 (65.0±13.9).

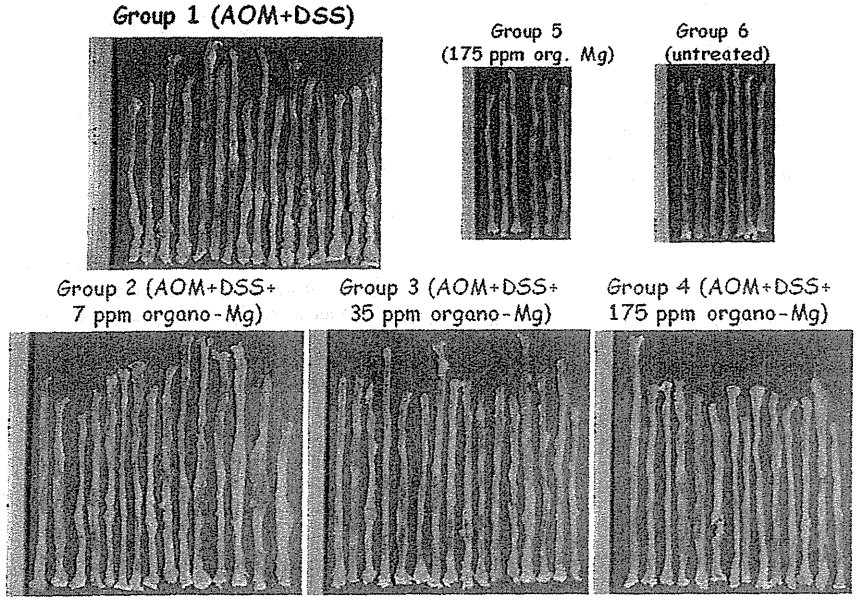

Fig. 1. Macroscopic views of the colon from the mice of groups 1–6 at the end of the study. Many tumors developed in the colon of mice in group 1 but number of colon tumors in groups 2–4 were less when compared with group 1. There were no tumor developed in the colon of mice in groups 5 and 6.

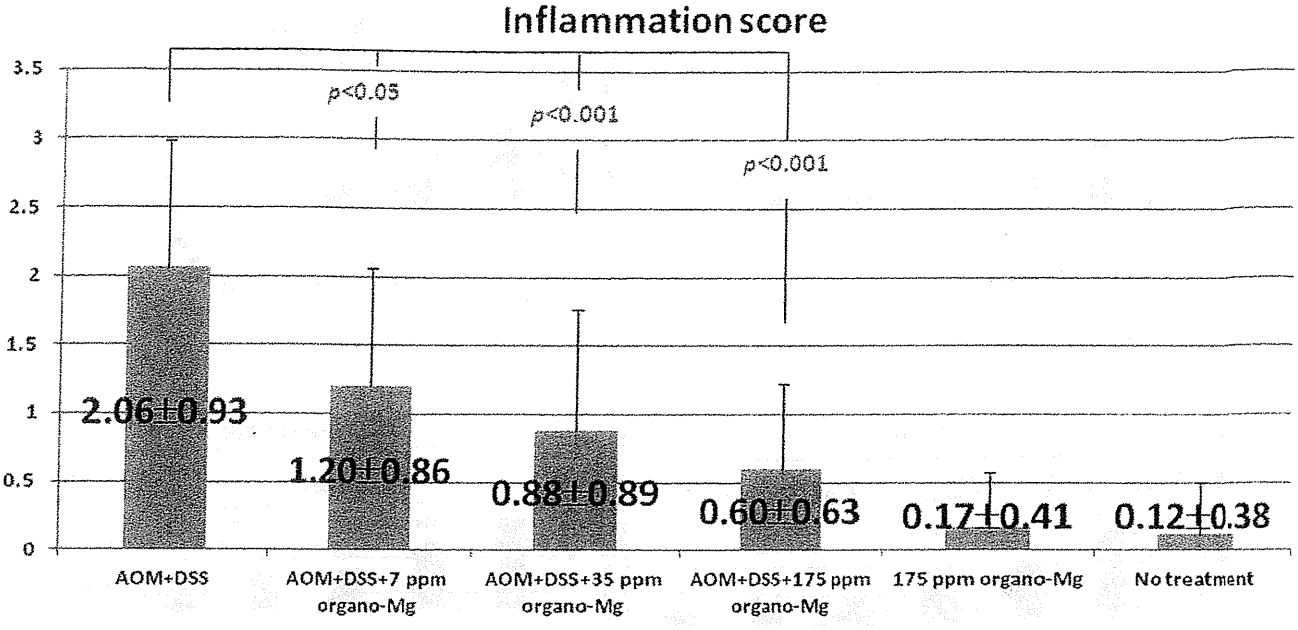

Fig. 3. Inflammation scores of colorectum in all groups. Administration of organo-Mg in drinking water significantly lowered the score of inflammation in the colorectum at 7 p.p.m. ($P < 0.05$), 35 p.p.m. ($P < 0.001$) and 175 p.p.m. ($P < 0.001$).

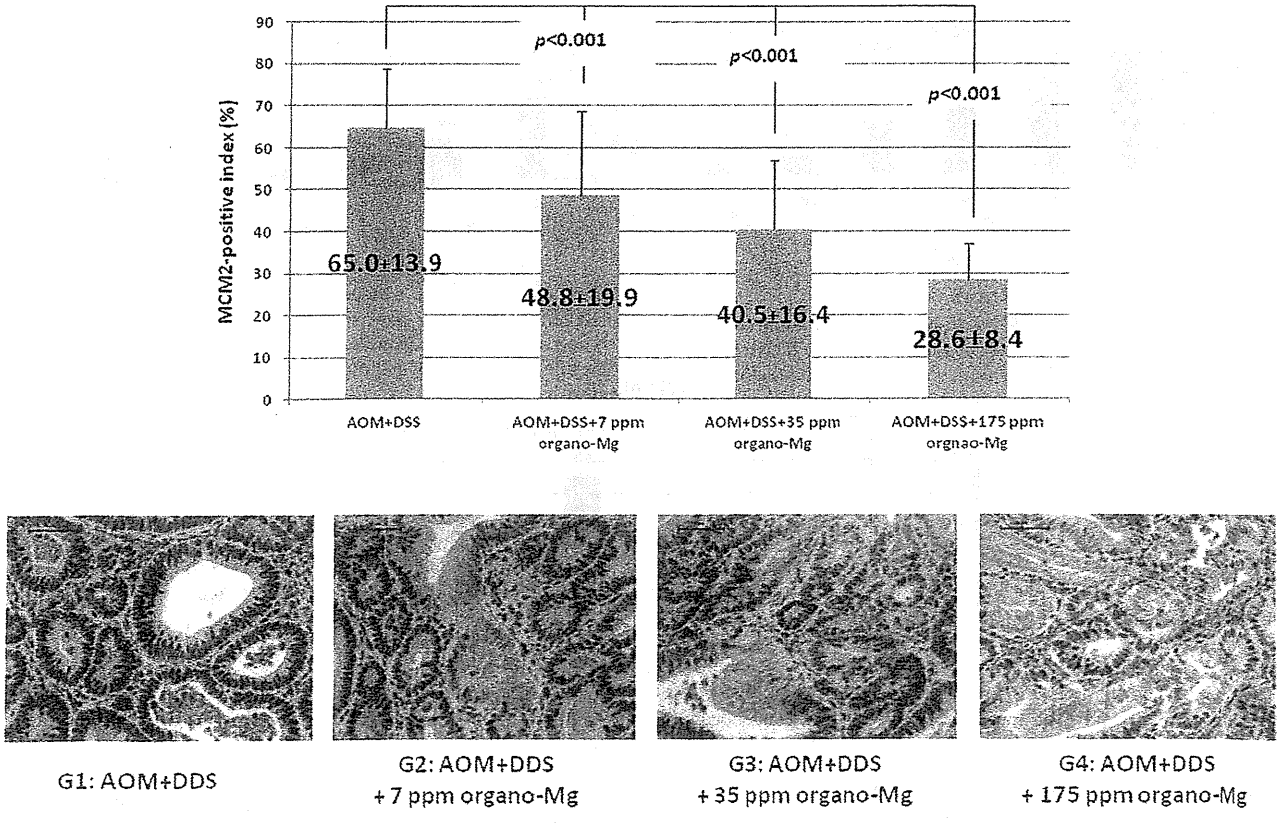

Fig. 4. The MCM2-positive indices (%) of adenocarcinoma cells. Administration with organo-Mg (group 2, $P < 0.001$: group 3, $P < 0.001$: group 4, $P < 0.01$) significantly lowered MCM2-positive indices of adenocarcinoma cells when compared with group 1. The MCM2-positive index (mean ± SD) of normal crypts ($n = 10$) was 6.20 ± 1.99.

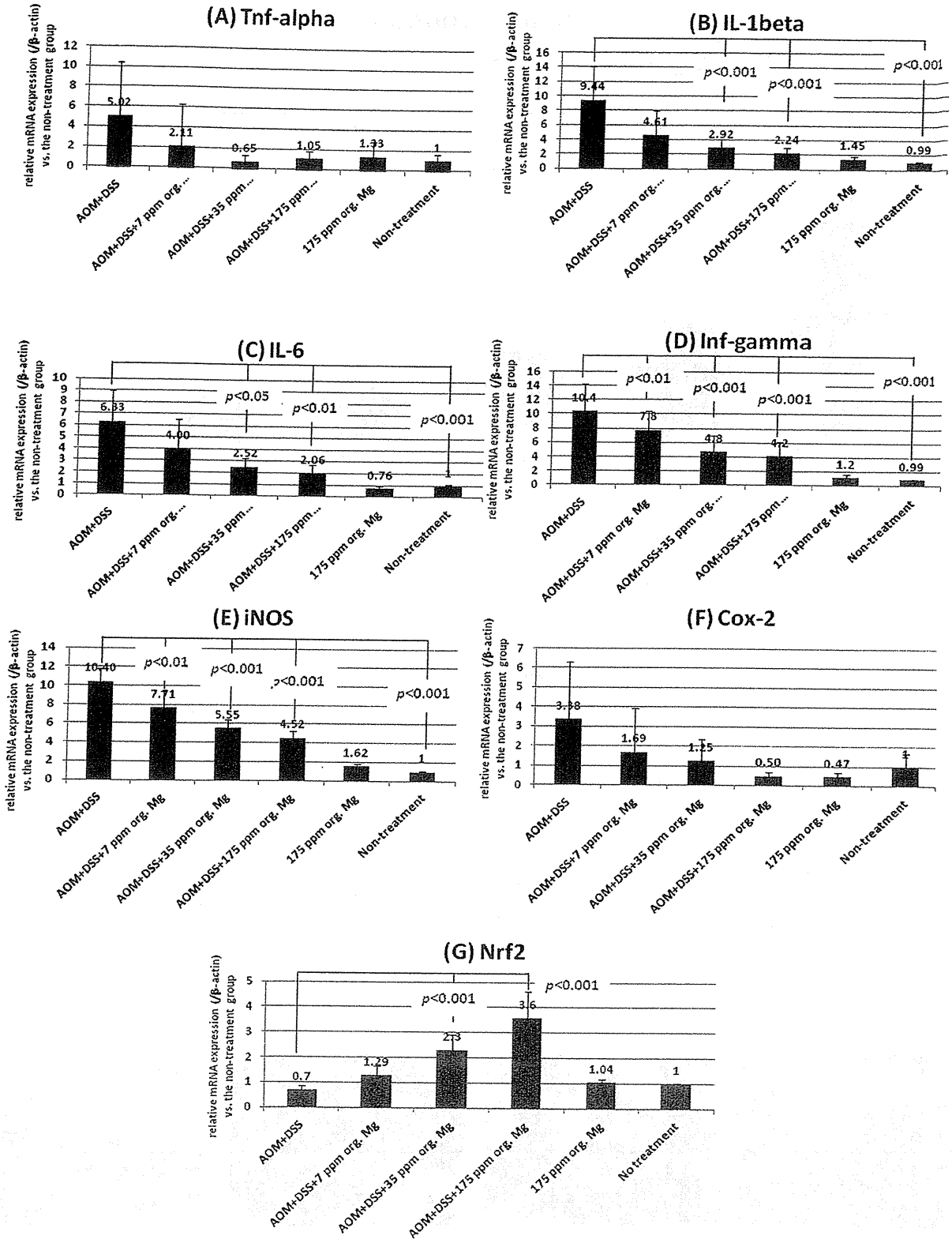

Fig. 6. The mRNA expression levels of (A) TNF-α, (B) IL-1β, (C) IL-6, (D) INF-γ, (E) iNOS, (F) COX-2 and (G) Nrf2 in lesion-free colonic mucosa of all the groups that were assessed by the quantitative real-time RT–PCR. Organo-Mg in the drinking water significantly lowered the expression level of IL-1β (35 and 175 p.p.m.), IL-6 (35 and 175 p.p.m.), iNOS (7, 35 and 175 p.p.m.) and IFN-γ (7, 35 and 175 ppm) when compared with the AOM and DSS group. The expression was normalized to β-actin mRNA expression. Samples were analyzed in triplicate. Data are mean ± SD from three independent assays ($n = 5$ from each group). Statistical analysis was performed by Kruskal–Wallis test. Ordinates are relative mRNA expression (β-actin) versus the non-treatment group.

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Low and Medium but Not High Doses of Green Tea Polyphenols Ameliorated Dextran Sodium Sulfate-Induced Hepatotoxicity and Nephrotoxicity

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Our previous study indicated that a diet containing a high dose (1%) of green tea polyphenols (GTPs) disrupted liver and kidney function *via* a reduction in antioxidant enzyme and heat shock protein (HSP) levels in both colitis and non-treated ICR mice. In the present study, we assessed the effects of 0.01%, 0.1%, and 1% dietary GTPs on liver and kidney physiological functioning in dextran sulfate sodium (DSS)-exposed and normal mice. GTPs at 0.01% and 0.1% significantly suppressed DSS-increased serum aspartate 2-oxoglutarate aminotransferase (AST) and alanine aminotransferase (ALT) levels. In contrast, GTPs at 1% increased kidney weight, serum creatinine levels, and thiobarbituric acid-reactive substances (TBARs) in both the kidney and the liver in normal mice, as compared with DSS-exposed mice. GTPs at 0.01% and 0.1% remarkably upregulated the expression of heme oxygenase-1 (HO-1) and heat shock protein 70 (HSP70) mRNA in the liver and kidney of mice exposed to DSS, whereas GTPs at 1% abolished it. Our results indicate that low and medium doses of GTPs have beneficial effects on DSS-induced hepatotoxicity and nephrotoxicity *via* upregulation of self-protective enzymes, while these effects disappeared at a high dose.

Key words: green tea polyphenols; colitis; hepatotoxicity; nephrotoxicity; self-protective enzymes

The inflammatory bowel disease (IBD), which includes the major chronic diseases ulcerative colitis (UC) and Crohn's disease (CD), is a chronic disorder of the intestinal tract characterized by excessive production of reactive oxygen species (ROS) and cytokines.[1)] As an experimental model, dextran sulfate sodium (DSS)-induced colitis in rodents is used in many laboratories including ours, due to convenient induction of intestinal inflammation.[2–4)] In addition, excess ROS generation in the gut microenvironment disrupts intestinal anti-oxidant systems, contributing to intestinal oxidative injury and initiating pro-inflammatory signaling.[5–7)]

The cellular stress response is an evolutionarily conserved defense mechanism characterized by transcriptionally controlled induction of the synthesis and accumulation of stress proteins, including heat shock proteins (HSPs), following exposure of cells to high temperatures and other environmental challenges including oxidative stress.[8)] In particular, a highly stress-inducible member of the 70-kDa family of HSP (HSP70) in cytosol functions as a predominant molecular chaperone.[9)] HSP70 has been found to be downregulated in actively inflamed mucosa of individuals with UC and CD.[10)] It is also important to note that the production of pro-inflammatory cytokines tumor necrosis factor-α and interferon-γ, which appears at increased levels in IBD, is also downregulated by HSP70, which inhibits the translation step.[5,11,12)] Collectively, inflammation-related signals in IBD might exacerbate colitis at least in part through downregulation of HSP70, thereby interfering with its vital role in maintaining intestinal homeostasis.

Green tea is a widely consumed beverage that contains characteristic polyphenolic constituents, generally known as green tea polyphenols (GTPs), which include (−)-epigallocatechin-3-gallate (EGCG), (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin.[13)] EGCG, the most abundant polyphenol, has been reported to have various preventive effects as to several chronic diseases, including cancer and disease.[14,15)] In addition, GTPs are strong antioxidants as against ROS and inducers of several antioxidant proteins, including heme oxygenases-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO1), which are regulated by activation of nuclear factor erythroid-2-related factor 2.[16,17)] On the other hand, we reported recently that a diet including 1% GTP damaged pro-inflammatory cytokines, aggravated colitis, and tended to promote colon carcinogenesis in DSS-exposed colons, while it decreas-

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Abbreviations: GTPs, green tea polyphenols; DSS, dextran sulfate sodium; AST, aspartate 2-oxoglutarate aminotransferase; ALT, alanine aminotransferase; TBARS, thiobarbituric acid-reactive substances; HO-1, heme oxygenase-1; HSP, heat shock protein

ed the activities of superoxide dismutase (SOD) and catalase in non-treated mice.[18] Furthermore, a 1% GTPs diet exacerbated kidney and liver functioning, presumably through downregulation of antioxidant enzymes and HSPs, in both normal mice and ones with DSS-induced colitis.[19] Lambert *et al.* found that EGCG at 1% induced hepatotoxicity, as demonstrated by increased formation of malonyldialdehyde and 4-hydroxynonenal.[20] In addition, several cases of hepatotoxicity in humans following the consumption of dietary supplements containing green tea extracts have been reported.[21] Thus, GTPs have both beneficial and harmful effects as to organ homeostasis in a dose-dependent manner.

The aim of the present study was to examine the effects of oral feeding of GTPs at various doses (0.01%, 0.1%, and 1%) on liver and kidney functioning and the expression of antioxidant enzymes and HSPs in both normal and DSS-treated ICR mice.

Materials and Methods

Chemicals. Extracted green tea polyphenols (HPLC graded GTPs containing 95% polyphenols) were purchased from LKT Laboratories (St. Paul, MN). High-performance liquid chromatography analysis of the green tea extracts revealed a percentage composition of 70% total catechins, 35% EGCG, and 3% caffeine. DSS, with a molecular weight of 36–50 kDa, was purchased from MP Biomedicals (Aurora, OH). The other chemicals and kits were from Wako Pure Chemical Industries (Osaka, Japan), unless specified otherwise.

Animals. Four-week-old male specific pathogen-free ICR mice (17–19 g) were purchased from Japan SLC (Shizuoka, Japan) and housed five per cage. All were fed rodent MF pellets (Oriental Yeast, Kyoto, Japan) were given fresh tap water *ad libitum*, and were kept at 22–26 °C at a relative humidity of 55–65% under a 12-hour light (06:00–18:00)/dark cycle for 6 d prior to experiments. The mice were treated in accordance with the Guidelines for the Use of Experimental Animals of Kyoto University, and the experimental protocol was approved by the Experimentation Committee (approval #21-42).

Experimental design. As shown in Fig. 1A, mice were randomly divided into eight groups: a non-treated group (group 1), GTP supplementation groups (group 2, 0.01% GTPs; group 3, 0.1% GTPs; group 4, 1% GTPs), a DSS-exposed group (group 5), and GTPs + DSS-exposed groups (group 6, 0.01% GTPs; group 7, 0.1% GTPs; group 8, 1% GTPs). Experimental colitis was induced in the DSS groups exposed to by administrating 5% DSS (w/v) through the water during the experimental period. Body weights and food intake for all groups were recorded each day until the end of the experiment. All the mice were euthanized by deep anesthesia with diethyl ether to determine the effects of dietary GTPs on DSS-induced colitis.

RNA extraction and reverse transcription PCR (RT-PCR) analysis. After isolation of the kidneys and livers, total RNA was prepared using Trizol (Invitrogen, Tokyo) as described in the manufacturer's protocol. For RT-PCR analysis, 1 μg of RNA was reverse transcribed with an RNA PCR kit (Takara, Shiga, Japan) with oligo dT-adaptor primer, as recommended by the supplier. PCR was done using a thermal cycler (PTC-0100; MJ Research, Cambridge, MA) with mouse hypoxanthine phosphoribosyl transferase (HPRT), HO-1, HSP70, and HSP90 primers.[19] The PCR products were subjected to electrophoresis in 3% agarose gels and were stained with 0.01% SYBR Gold stain (Molecular Probes, Eugene, OR). Band intensities were quantified by NIH image, and an absence of PCR saturation was confirmed.

AST, ALT, and creatinine measurements. Blood was collected from the inferior vena cava, and serum was obtained by centrifugation at 3,000 × *g* for 10 min at 4 °C for analyst of biomarkers. AST and ALT (Wako Pure Chemical Industries) were used as biochemical markers, of hepatoxicity and were measured using by commercially available spectrophotometric assay ($\lambda_{max} = 555$ nm) following the manufacturer's instructions. Creatinine was quantified by means of commercial kits (Wako Pure Chemical Industries, Osaka).

Lipid peroxidation, as determined by thiobarbituric acid-reactive substances (TBARs). The kidney and liver samples (about 25 mg each) were homogenized in 250 μL of RIPA buffer (25 mm Tris–HCl pH 7.6, 150 mm NaCl, 1% NP-40, 1% deoxycholic acid, and 0.1% sodium dodecyl sulfate) on ice. The homogenate was centrifuged at 1,600 × *g* for 10 min at 4 °C and the supernatant was used in the assay. Lipid peroxidation in the kidney and liver was assessed by measuring TBARs using a TBARS Assay kit (Cayman Chemical, Ann Arbor, MI).

Statistical analysis. Differences in survival were analyzed by χ^2 analysis. Results were expressed as mean ± SD for each group of 3–7 mice. After examining equality by Levene's test, if there was a significant difference ($p < 0.05$), each value was converted to the logarithmic value for ANOVA. Data were analyzed by two-way ANOVA to determine the effects of DSS treatment (D), GTP administration (G), and their interaction (D × G). Tukey's test was used to determine the significance of differences were resulting from multiple comparisons among the groups, and differences considered to be significant at $p < 0.05$.

Results

General observations

Although no mice in groups 1–4 died during the 6 d of observation, the survival rates in groups 5–8 decreased on day 6 (Fig. 1B). In particular, group 8 exhibited a drastic decrease in survival rate, by 80%, on day 6 as compared to group 1. Mean body weights in groups 5–8 were lower than that group 1, whereas GTP supplementation in normal mice (groups 2–4) resulted in no marked differences (Fig. 1C). Data for water and food intake showed tendencies similar to those for body weight (Fig. 1D, E).

Effects of GTPs on colon, spleen, kidney, and liver weights

Colorectal shortening reflects the extent of colonic damage in DSS-exposed mice.[22] As shown in Fig. 2A, colorectal lengths in groups 5–8 were significantly shortened as compared to group 1, while GTPs supplementation in normal mice had no effect (group 1 *vs.* 2, 3, 4). In addition, we observed that colorectal length in group 8 was significantly shortened as compared group 5 (Fig. 2A). Spleen weight in group 5 was significantly higher (2-fold) that in group 1 (Fig. 2B). On the other hand, GTP supplementation (groups 2–4, and 6–8) did not affect weight as compared groups 1 and 5 (Fig. 2B). Kidney weight in group 8 was notably increased as compared to group 5 (Fig. 2C), while those in groups 1–7 did not vary significantly. In contrast, liver weight was nearly constant in all groups (Fig. 2D).

Kidney and liver function parameters

Serum AST and ALT levels, which reflect hepatic function, were also measured. Group 5 (AST, 324.03 ± 25.04 U/L; ALT, 71.76 ± 15.31 U/L) showed a marked increase in both parameters as compared to group 1 (AST, 98.93 ± 15.6 U/L; ALT, 16.25 ± 3.52 U/L). Low and medium doses of GTPs (group 6, 281.75 ± 14.57 U/L; group 7, 279.92 ± 15.22 U/L) significantly decreased AST levels, by 13% and 14% respectively, while a high dose (group 8, 326.05 ± 32.52 U/L) was ineffective. (Fig. 3A, B). In addition, group 6 (52.57 ±

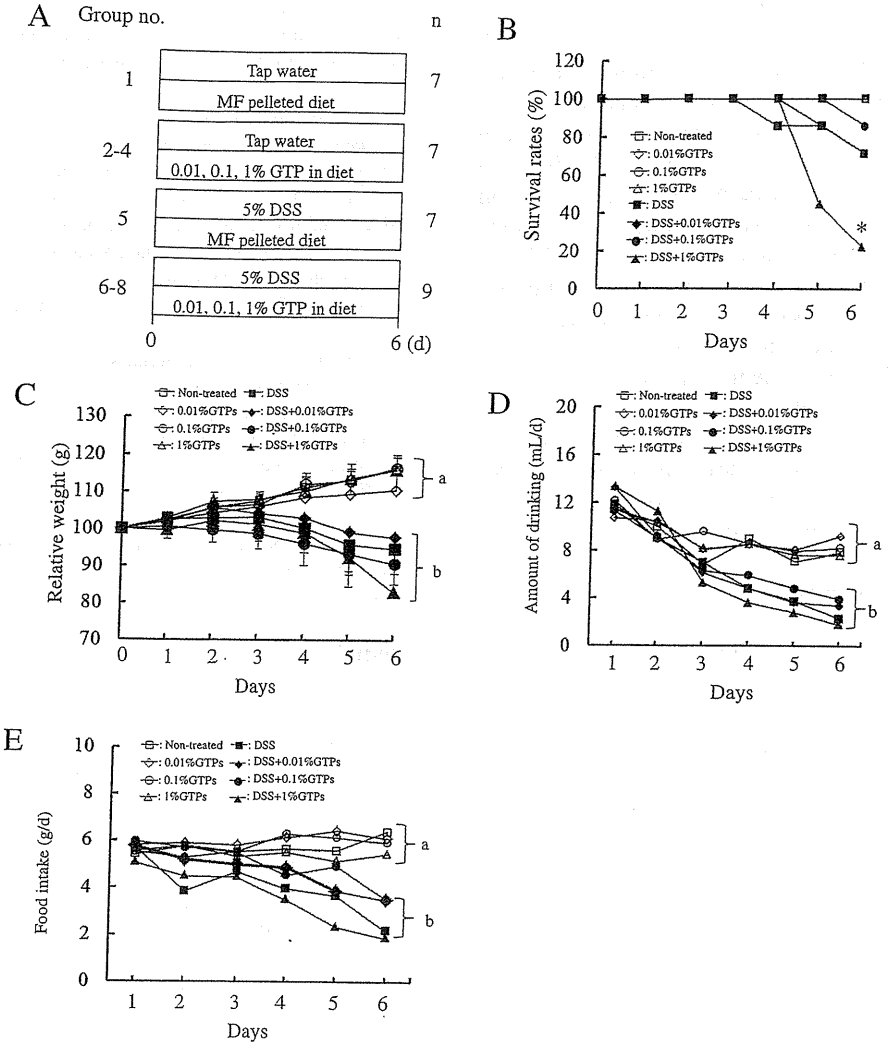

Fig. 1. Experimental Groups.

The non-treated group (group 1 (□), n = 7) was given tap water and a basal diet *ad libitum*, made fresh every day, for 6 d. The GTPs groups (groups 2 (◇), 3 (○), 4 (△); n = 7) were given tap water and fed a diet containing 0.01%, 0.1%, or 1% GTPs respectively, for 6 d. The DSS group (group 5 (■), n = 7) was fed a basal diet and given 5% DSS (w/v) in tap water for 6 d to induce colitis. The 0.01%, 0.1%, and 1% GTPs + 5% DSS groups (groups 6 (◆), 7 (●), 8 (▲) respectively; n = 7–9) were fed a diet containing those GTP concentrations and given 5% DSS (w/v) in tap water for 6 d. The effects of DSS and/or GTP supplementation on survival (B), relative body weight (C), water intake (D), and food intake (E), were determined. Data are means ± SD. $^*p < 0.05$ by χ^2 test. Differences were considered to be significant at $p < 0.05$.

7.24 U/L) and group 7 (44.4 ± 8.67 U/L) significantly decreased in ALT levels by 27% and 38% respectively, as compared to group 5 (71.76 ± 15.31 U/L). Subsequently, we measured serum creatinine levels as a biomarker of renal function.[23] DSS exposure (group 5) did not affect the level of serum creatinine, whereas that in group 8 doubled as compared with group 5 (Fig. 3C). Next we compared TBARS levels, a reliable indicator of lipid peroxidation that might be related to oxidative tissue damage.[12] As shown in Fig. 3D and E, both hepatic and renal TBARS levels in group 8 were significantly higher than those in group 5. Likewise, those levels in both the liver and the kidney in group 4 were also markedly increased as compared group 1 (Fig. 3D, E). Low-dose GTPs did not affect TBARS levels in the normal or the colitis mice.

Effects of GTPs on the expression levels of antioxidant enzymes and HSPs

We also determined whether DSS and/or GTPs supplementation would affect the expression levels of HO-1, HSP70, and HSP90 in the kidney and the liver by semi-quantitive RT-PCR (Fig. 4A). Both renal and hepatic HO-1 mRNA expressions in groups 6 and 7 were significantly increased as compared with group 5. In contrast, high-dose GTPs (group 8) abolished them (Fig. 4B). Similarly, renal HO-1 mRNA levels were significantly increased by supplementation with GTPs at 0.01% and 0.1%, but not at 1% (Fig. 4B, left). On the other hand, the hepatic HO-1 mRNA level was not different among groups 1, 2, and 3, while that in group 4 was significantly changed (Fig. 4B, right).

Renal HSP70 expression in groups 2–4 did not change as compared with group 1, whereas it decreased drastically in group 5 (Fig. 4C, left panel). The renal HSP70 level in group 7 was significantly higher than in group 5 (Fig. 4C, left panel). Also, the hepatic HSP70 expression levels in groups 6 and 7, but not in group 8, were significantly increased as compared with group 5 (Fig. 4C, right panel). Likewise, those in groups 2 and 3 were remarkably higher than that in group 1, whereas the level in group 4 was decreased drastically (Fig. 4C). Both renal and hepatic HSP90 expression decreased markedly, in a dose-dependent manner, in groups 2–4,

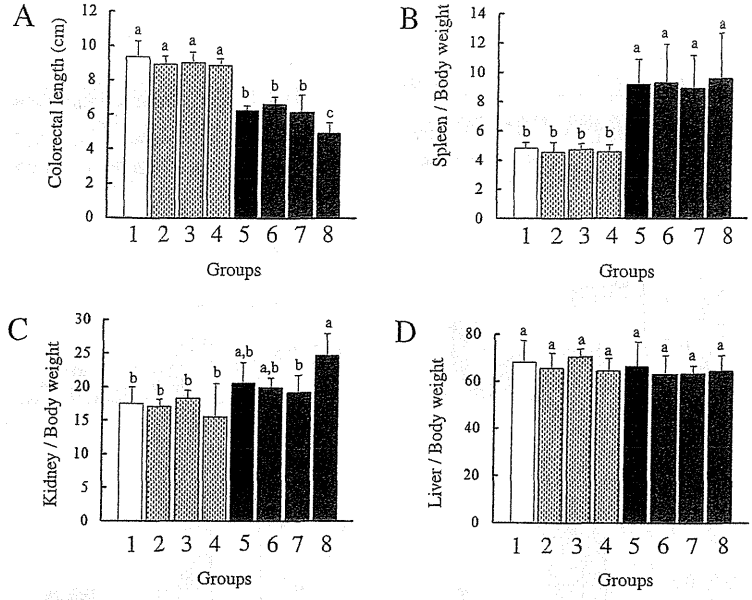

Fig. 2. Effects of DSS and/or GTP Supplementation on Symptomatic Changes in Several Organs.
Colon length (A), spleen weight (B), kidney weight (C), and liver weight (D) were determined. Data are means ± SD. Differences were considered to be significant at $p < 0.05$.

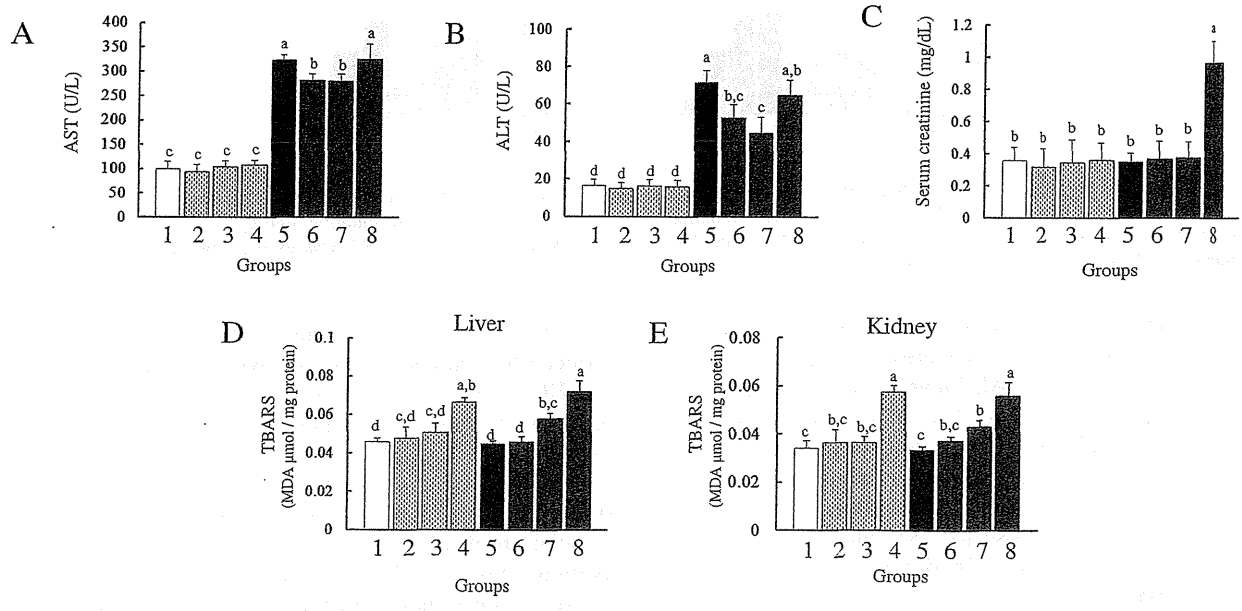

Fig. 3. Effects of DSS and/or GTP Supplementation on Hepatotoxicity and Renal Toxicity.
Blood was collected and serum separated for determination of serum AST (A), ALT (B), and creatinine (C) levels. The kidneys and livers were homogenized using RIPA buffer, and, then the supernatants were separated for measurement of TBARS levels in the liver (D) and kidney (E). Data are means ± SD. Differences were considered to be significant at $p < 0.05$.

and similar tendencies were seen for the DSS-exposed mice (Fig. 4D).

Discussion

GTPs and EGCG have a variety of beneficial health functions, including preventive effects on inflammation and carcinogenesis, and have been reported to have no serious adverse effects.[14,15] However, several studies have revealed that excess intake of green tea supplements induced hepatotoxicity in both rodents and humans.[20,21,24] Similarly, our group has also reported that a 1% GTP diet enhanced pro-inflammatory cytokines, aggravated colitis, and tended to promote colon carcinogenesis in DSS-exposed colons.[18] On the other hand, a low-dose GTP (0.1–0.25%) diet had a tendency to improve both ulcers and inflammation in a colitis model. Furthermore, we reported recently that a 1% GTP diet damaged the kidney and the liver functioning *via* downregulation of antioxidant enzymes and HSPs in DSS-induced colitis,[19] but the molecular mechanisms are poorly understood, and the effects of low- and medium-dose GTP diets on these functions have yet to be fully elucidated. Therefore, our aim was to determine the effects of oral feeding of GTPs at various doses on the liver and the kidney functions in DSS-induced colitis.

Several groups have reported that intragastric administration of high-dose EGCG caused hepatotoxicity in

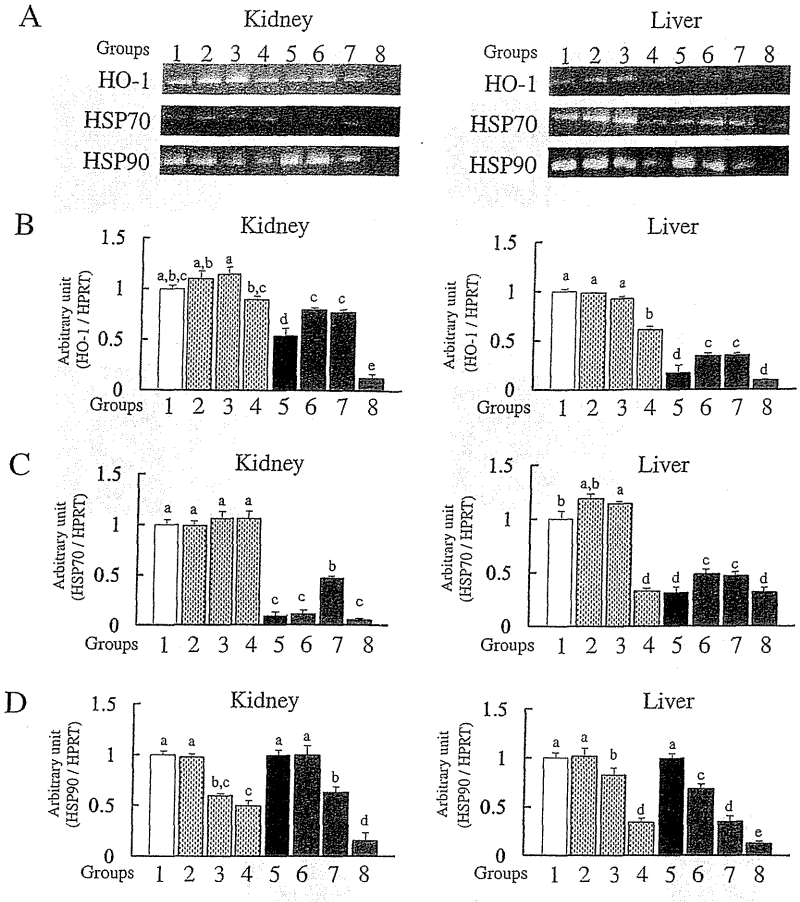

Fig. 4. Effects of DSS and/or GTP Supplementation on Antioxidant Enzyme mRNA Levels in Mouse Kidneys and Livers.
HO-1, HSP70, and HSP90 mRNA expression was determined by RT-PCR (A), and representative findings presented. Densitometric quantification of HO-1 (B), HSP70 (C), and HSP90 (D) mRNA was done using NIH Image. HPRT was used as internal control. Data are means ± SD. Differences were considered to be significant at $p < 0.05$.

mice,[20,25] while various doses of GTPs in the diet did not damaged liver function in the present study, as determined by serum AST and ALT levels (Fig. 3A, B). These contrasting results may have been due to differences in the experimental conditions imposed. We found that 1% GTPs dramatically increased kidney weight and serum creatinine, the most reliable biomarkers of nephropathy,[19,23,26] in mice expose to DSS, but not in normal mice (Figs. 2C, 3C). These findings confirm previous reports showing that both green tea extracts and DSS become widely distributed throughout a variety of organs in mice, including the liver and kidney,[27,28] but EGCG is metabolized through methylation, glucuronidation, and sulfation under normal physiological conditions, and then excreted in the urine.[29,30] Thus low- and medium-dose GTPs are metabolized and excreted in the urine, while a high dose can accumulate in various organs of normal and DSS-exposed mice, including the liver and kidney, inducing oxidative damage. Along similar lines, 1% GTPs drastically increased the level of TBARS in the kidney and liver, while 0.01–0.1% GTPs did not change those in normal or DSS-exposed mice (Fig. 3D, E). Therefore high-dose, but not low- or medium-dose, GTPs more strongly induced oxidative stress *via* auto-oxidation by producing ROS in the normal and DSS-exposed mice, which escapes from the inactivation processes by metabolism.

Oxidative stress is accelerated by a combination of ROS generation and impaired antioxidant capacity.[31–33] Our group has reported that a high dose of EGCG reduced the expression of anti-oxidant enzymes, including HO-1, SOD, and catalase.[18,19] In accordance with this observation, 1% GTPs abolished HO-1 mRNA expression in the kidney and liver, whereas 0.01–0.1% GTPs in the diet significantly increased mRNA expression (Fig. 4A, B). These findings suggest a mechanal association of that anti-oxidative property with low and medium doses in normal and DSS-induced mice. In addition, EGCG has been found to enhance Nrf2-Keap1 signaling *in vivo* and to increase the expressions of other anti-oxidant enzymes, including NQO-1.[34] Hence we speculate that low and medium doses of GTPs protect against oxidative stress by increasing anti-oxidant enzymes, whereas a high dose causes oxidative stress *via* downregulation of antioxidant enzymes, leading to hepatic and renal dysfunction.

The HSP family functions as a redox sensor and represents an endogenous protective mechanism against oxidative stress.[35,36] Accordingly, in the present study, we found that renal and hepatic HSP70 mRNA expression was dramatically suppressed by a high dose of GTPs in the diet, while low and medium doses increased expression in normal and DSS-exposed mice (Fig. 4C), although protein levels remain to be examined. Hu *et al.* have reported that downregulation of HSP70 was associated with IBD development.[11] Thus a significant increase in HSP70 expression due to low- and medium-dose GTPs, but not a high dose, might protect

against oxidative stress. Importantly, HSP90 in the kidney and liver was identified as a chaperone protein that is downregulated by GTPs given at medium and high doses in non-treated mice (Fig. 4D). HSP90 is the most abundant molecular chaperone and it plays pivotal roles in inducing the expression of other HSP genes, resulting in organ homeostasis under various stresses.[37–39] Thus high-dose GTPs may affect hepatic functioning *via* HSP90 downregulation in healthy individuals.

Taken together, our findings suggest that low and medium doses of GTPs have beneficial effects in DSS-induced hepatotoxicity and nephrotoxicity *via* upregulation of anti-oxidant enzymes and HSPs. In addition, attenuation of these levels can increase disease activity in DSS-induced colitis. Finally, our results provide insight into the molecular mechanisms underlying the potential toxicity of GTPs.

Acknowledgments

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The pathological significance of Notch1 in oral squamous cell carcinoma

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Notch signaling has been reported to be involved in several types of malignant tumors; however, the role and activation mechanism of Notch signaling in oral squamous cell carcinoma (OSCC) remains poorly characterized. The purpose of this study was to elucidate the pathological significance of Notch signaling and its activation mechanism in the development and progression of OSCC. In this study, we showed that the expression of Notch1 and intracellular Notch domain (NICD) are upregulated in OSCCs. In addition, Notch1 and NICD were found to be characteristically localized at the invasive tumor front. TNF-α, a major inflammatory cytokine, significantly activated Notch signaling *in vitro*. In a clinicopathological analysis, Notch1 expression correlated with both the T-stage and the clinical stage. Furthermore, loss of Notch1 expression correlated with the inhibition of cell proliferation and TNF-α-dependent invasiveness in an OSCC cell line. In addition, γ-secretase inhibitor (GSI) prevented cell proliferation and TNF-α-dependent invasion of OSCC cells *in vitro*. These results indicate that altered expression of Notch1 is associated with increased cancer progression and that Notch1 regulates the steps involved in cell metastasis in OSCC. Moreover, inactivating Notch signaling with GSI could therefore be a useful approach for treating patients with OSCC.

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KEYWORDS: invasion; Notch signaling; oral squamous cell carcinoma; TNF-α; proliferation

Oral cancer is one of the most common cancers,[1] and the survival rate of patients with oral cancer has not improved despite improvements and innovations in diagnostic techniques and treatments.[2] It is thought that most, if not all, cases of oral squamous cell carcinoma (OSCC) are preceded by a period during which the affected epithelium shows evidence of epithelial dysplasia, although this may not always be clinically apparent.[3] Histological criteria to assess cellular and tissue changes (dysplasia) have been defined by the World Health Organization[4] and are related to alterations in the kinetics of cellular proliferation and the maturation of the epithelium.[5] A better understanding of the molecular mechanisms underlying the development of OSCC may add to the treatment and prognosis of this disease.

The Notch signaling pathway has an important role in cellular patterning and differentiation during development.[6] Notch signaling has been highly conserved through the evolution from worm to man. Mammals have four Notch receptors (Notch1–4) that are transmembrane receptors harboring an extracellular domain responsible for the binding of their specific ligands and an intracellular domain involved in transcriptional regulation. Notch receptors can receive signals from neighboring cells that express transmembrane-type ligands such as Delta-like ligand and Jagged (JAG).[7] Ligand binding to Notch receptors leads to the activation of common intracellular signaling pathways, which results in the cleavage of the intracellular Notch domain (NICD). NICD translocates into the nucleus and heterodimerizes with CSL/CBF-1/RBP-Jκ. The CSL/CBF-1/RBP-Jκ–NICD complex is activated by the Mastermind family of coactivators and transactivates target genes such as mammalian hairly enhancer of split (Hes) and Hey family members.[8]

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