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 magnetic 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.

Abbreviations: ABL, anaphase bridging index; AOM, azoxymethane; 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. 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 azoxymethane (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 sulfate sodium (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 colorectal 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 assayed 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.
Materials and methods

Chemicals
Organ-Mg was prepared by mixing magnesium oxide (0.22 g), citric acid (0.55 g), malic acid (0.55 g) and glycine (0.22 g) in the Tateho Chemical Industries Co., Ltd. (Ako City, Hyogo, Japan). X-ray diffraction of organo-Mg was performed in the Air Water Inc. (Sapporo, Japan). Organ-Mg (1.54 g) contained 132 mg Mg. AOM was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). DSS with a molecular weight of 36,000–50,000 was obtained from MP Biomedicals, LLC (Aurora, OH, USA). DSS 1.5% (w/v) was prepared just before use to induce colitis.

Animals
Five week old male Crl:CD-1 (ICR) mice were purchased from Japan SLC, Inc. All animals were housed in plastic cages (3–5 mice/cage) and had free access to tap water and a basal diet, CE-2 (CLEA Japan, Inc., Tokyo, Japan). The animals were kept in an experimental animal room under controlled conditions of humidity (50 ± 10%), light (12/12 h light/dark cycle) and temperature (23 ± 2°C). The experimental and study design were approved by the Committee of Gifu University Animal Facility under the Institutional Animal Care guideline. All handling and procedures were carried out in accordance with the appropriate Institutional Animal Care Guidelines.

A total of 105 male ICR (4 weeks old) mice were divided into five experimental groups and one control group. Experimental drinking water was prepared by dissolving organo-Mg in distilled water at three dose levels of 7, 35 and 175 ppm.

Animal experiment
The experimental and study design were approved by the Committee of Gifu University Animal Facility under the Institutional Animal Care guideline. All handling and procedures were carried out in accordance with the appropriate Institutional Animal Care Guidelines.

A total of 105 male ICR (4 weeks old) mice were divided into five experimental groups and one control group. Experimental drinking water was prepared by dissolving organo-Mg in distilled water at three dose levels of 7, 35 and 175 ppm. Each large bowel was cut open longitudinally along the main axis and gently washed out by physiologic saline and the length from the ileocecal junction to the anal verge was measured. After the large bowels were cut open longitudinally along the main axis and gently washed with saline, scraped colonic mucosa tissue was dipped into the RNAlater solution (Applied Biosystems/Ambion, Life Technologies Japan, Ltd., Tokyo, Japan). At sacrifice, the large bowel was removed and the length was measured. The body weight gains by mice in all of the six groups were similar.

Colitis was graded according to the following morphological criteria (26): showing normal appearance (grade 0); shortening and loss of the mucosal layers on hematoxylin and eosin–stained sections made from paraflin-embedded blocks. Colonic tumors were diagnosed according to criteria established in a prior study (19). The number and density of mucosal ulcers on hematoxylin and eosin–stained sections was also measured.

Mitotic index and anaphase bridging index of adenocarcinoma cells
To examine the effects of organo-Mg in drinking water on chromosomal instability (24,25) in adenocarcinoma cells, the anaphase bridging index (ABI) was determined on hematoxylin and eosin–stained sections. The numbers of mitoses and anaphase bridging were counted in >100 cancer cells each from five adenocarcinomas each from groups 1 through 4. The mitotic index (MI; number of mitoses per cancer cells) and ABI (number of anaphases with bridging per mitoses) were expressed as percentages (mean ± standard deviation (SD)).

Total RNA extraction and quantitative real-time RT–PCR
Total RNA was extracted from colonic mucosa using the RNeasy Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer’s protocol. The complementary DNA was then synthesized from total RNA (0.2 µg) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems Japan Ltd., Tokyo, Japan). Quantitative real-time PCR analysis of individual complementary DNA was performed with ABI Prism 7500 (Applied Biosystems Japan Ltd.) using TaqMan Gene Expression Assays (Applied Biosystems Japan Ltd.; TFN-α, Mm00443258_m1; IL-1β, Mm00434228_m1; IL-6, Mm00446190_m1; INF-γ, Mm0081778_m1; COX-2 (Ptgs2), Mm00478374_m1; iNOS (Nos2), Mm00440485_m1 and IFN-β, Mm00807939_s1). The sense and antisense primers Nrf2 mRNA were 5′-CGGGCATGCTTTCTGCTCA-3′ and 5′-GTGGGACAATGTCCATAC-3′, respectively. 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. Each assay was performed triplicate and the average was calculated.

Effects of organo-Mg on the growth of human colorectal adenocarcinoma cell line DLD-1
DLD-1 human colorectal cancer cells were purchased from American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum. Fetal bovine serum and charcoal-dextran stripped fetal bovine serum were purchased from Biowest (Ringmer, UK). WST-1 reagent was obtained from Roche Diagnostics (Mannheim, Germany). The cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO2. Cell proliferation was determined on hematoxylin and eosin–stained sections. The numbers of mitoses per cancer cells and ABI (number of anaphases with bridging per mitoses) were expressed as percentages (mean ± SD).

Results

General observation
All animals remained healthy throughout the experimental period. The body weight gains by mice in all of the six groups were similar.
Organo-Mg and inflammation-related colorectal carcinogenesis
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 1). 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 (Figure 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 addition, organo-Mg
exposure to mice at 175 p.p.m. in the drinking water significantly
diminished the incidences of colorectal adenoma and adenocarci-
noma ($P < 0.01$ for both the lesions). The administration of 7 p.p.m.

**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 inflamma-
tion 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 adenocarcin-
obras 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.](https://academic.oup.com/carcin/article-abstract/34/2/361/2464140)
The effects of organo-Mg on the MI and ABI

The exposure to organo-Mg affected the number of mitosis (Figure 5A) and anaphase bridging (Figure 5B) in adenocarcinomas. As illustrated in Figure 5A, drinking with organo-Mg significantly decreased the MI in groups 3 (21.1 ± 7.3, \( P < 0.01 \)) and 4 (19.2 ± 4.4, \( P < 0.01 \)) compared with group 1 (27.0 ± 7.2). The treatment also lowered the ABI in group 3 (2.41 ± 0.91, \( P < 0.05 \)) and group 4 (1.67 ± 0.78, \( P < 0.01 \)) compared with group 1 (3.57 ± 2.06) as shown in Figure 5B.

Expressions of inflammatory enzyme and cytokine genes in colonic mucosa

At week 4, we assayed mRNA levels of TNF-\( \alpha \), IL-1\( \beta \), IL-6, IFN-\( \gamma \), iNOS, COX-2 and Nrf2 in the non-lesional colonic mucosa of mice in groups 1 through 6 by quantitative real-time RT–PCR. AOM and DSS treatment increased mRNA expression of TNF-\( \alpha \), IL-1\( \beta \), IL-6, IFN-\( \gamma \), iNOS and COX-2 and lowered mRNA expression of Nrf2 when compared with the untreated group (Figure 6A–G). When given organo-Mg (35 and 175 p.p.m.) to mice, mRNA expression of IL-1\( \beta \) (Figure 6B, \( P < 0.001 \) at both doses), IL-6 (Figure 6C, \( P < 0.05 \) at 35 p.p.m. and \( P < 0.01 \) at 175 p.p.m.), IFN-\( \gamma \) (Figure 6D, \( P < 0.001 \) at both doses) and iNOS (Figure 6E, \( P < 0.001 \) at both doses) were significantly decreased when compared with the AOM and DSS group. Organo-Mg treatment even at the lowest dose of 7 p.p.m. was able to lower significantly IFN-\( \gamma \) (\( P < 0.01 \)) and iNOS (\( P < 0.01 \)) mRNA expression. Alterations in the mRNA expression of TNF-\( \alpha \) (Figure 6A) and COX-2 (Figure 6F) by organo-Mg treatment were not significant.

As to Nrf2 mRNA expression, organo-Mg treatment at 35 and 175 p.p.m. significantly increased the expression (\( P < 0.001 \) at both doses, Figure 6G).

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment (No. of mice at week 16)</th>
<th>Inflammation score</th>
<th>Mucosal ulcer</th>
<th>Dysplastic crypts (high grade)</th>
<th>Adenoma (AD)</th>
<th>Adenocarcinoma (ADC)</th>
<th>Total tumors (AD + ADC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AOM/DSS (16)</td>
<td>2.06±0.93(^a)</td>
<td>81% ± 1.69±1.20</td>
<td>100% ± 3.75±2.14</td>
<td>81% ± 2.31±1.92</td>
<td>88% ± 4.00±2.19</td>
<td>94% ± 6.31±3.20</td>
</tr>
<tr>
<td>2</td>
<td>AOM/DSS/7 p.p.m. organo-Mg (15)</td>
<td>1.20±0.86(^b)</td>
<td>67% ± 1.07±0.96</td>
<td>80% ± 2.67±1.63</td>
<td>73% ± 1.20±1.26</td>
<td>73% ± 2.00±1.93</td>
<td>80% ± 3.20±2.68</td>
</tr>
<tr>
<td>3</td>
<td>AOM/DSS/35 p.p.m. organo-Mg (16)</td>
<td>0.88±0.89(^c)</td>
<td>44% ± 0.69±0.87(^e)</td>
<td>81% ± 2.00±1.59(^e)</td>
<td>56% ± 1.06±1.29</td>
<td>81% ± 1.81±1.42(^e)</td>
<td>81% ± 2.88±2.31(^e)</td>
</tr>
<tr>
<td>4</td>
<td>AOM/DSS/175 ppm organo-Mg (15)</td>
<td>0.60±0.63(^c)</td>
<td>47% ± 0.53±0.64(^e)</td>
<td>80% ± 1.53±1.46(^e)</td>
<td>27% ± 0.47±0.92(^e)</td>
<td>47% ± 0.80±1.01(^e)</td>
<td>47% ± 1.27±1.71(^e)</td>
</tr>
<tr>
<td>5</td>
<td>175 ppm organo-Mg (6)</td>
<td>0.170±.41</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>None (7)</td>
<td>0.120±.38</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

\(^a\)Mean ± SD.
\(^b\)Significantly different from group 1 (\( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \)) by one-way analysis of variance with Tukey’s multiple comparisons test.
\(^c\)Significantly different from group 1 (\( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \)) by one-way analysis of variance with Tukey’s multiple comparisons test.
\(^d\)Significantly different from group 1 (\( P < 0.05 \) and \( P < 0.01 \)) by Fisher’s exact probability test.
\(^e\)Significantly different from group 1 (\( P < 0.05 \) and \( P < 0.01 \)) by Fisher’s exact probability test.
\(^f\)Significantly different from group 1 (\( P < 0.05 \), \( P < 0.01 \), and \( P < 0.001 \)) by one-way analysis of variance with Tukey’s multiple comparisons test.
Organo-Mg and inflammation-related colorectal carcinogenesis

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.
Antiproliferative effect of organo-Mg on DLD-1 cells

Cell viabilities after organo-Mg/ml medium were as follows in DLD-1 cells: 1 µg, 103.0 ± 2.3%; 10 µg, 102.6 ± 1.0%; 100 µg, 87.6 ± 6.5%; 1000 µg, 84.7 ± 1.2%. The vehicle alone (water) showed no effect on cell proliferation in cancer cells. Adding 1 and 10 µg/ml concentrations of organo-Mg in the medium did not affect the growth of DLD-1 cells. However, 100 and 1000 µg/ml of organo-Mg slightly lowered the growth of DLD-1 cells and the inhibition by 1000 µg/ml organo-Mg was statistically significant (P < 0.01) when compared with 0 µg/ml organo-Mg.

Discussion

In this study, we demonstrated that organo-Mg consumption in drinking water at three dose levels (7, 35 and 175 p.p.m.) significantly inhibited AOM/DSS-induced colorectal carcinogenesis, a model for human colon cancer, in male ICR mice. Even the low dose (7 p.p.m.) of organo-Mg significantly inhibited the development of adenocarcinomas induced by AOM and DSS. The treatment with organo-Mg resulted in reduction of the MCM2-positive index, MI and ABI in the colonic epithelial malignancies at week 16. Organo-Mg in drinking water also affected mRNA expression of certain proinflammatory cytokines (TNF-α, IL1-β and IL-6), inducible inflammatory enzymes (COX-2 and iNOS) and Nef2 in the colonic mucosa. These effects of organo-Mg may contribute to its suppression effects on AOM/DSS-induced colorectal carcinogenesis in mice. Our results confirmed utility of Mg for prevention of CRC development in humans (8,9) and our earlier experimental study (10).

The effects of organo-Mg on the ABI in the current study are interesting because human UC and UC-associated CRC have high frequency of chromosomal instability (CIN) when compared with colitis other than inflammatory bowel disease and sporadic CRC (15,16). Inflammation in several tissues increases genetic instability, including CIN and microsatellite instability, that contributes to colorectal carcinogenesis, especially colitis-associated CRC development. CIN results in abnormal segregation of chromosomes and abnormal DNA content (aneuploidy). As a result, loss of chromosomal material (loss of heterozygosity) often occur, such as APC and p53. A high frequency of microsatellite instability and p53 mutations are detected in UC patients even whose colonic mucosa was negative for dysplasia (28,29). Experimentally, CIN in adenocarcinomas induced by AOM and DSS was quite high (30), whereas the one induced by AOM alone did not show CIN (31). Thus, the AOM/DSS model (19) used in this study is useful for investigating pathogenesis and chemoprevention of colitis-associated colorectal carcinogenesis.

Several epidemiological studies have provided evidence that a correlation exists between dietary Mg and various types of cancer. High levels of Mg in drinking water protect against oesophageal and liver cancer (32,33). Mg concentration in drinking water and/or other dietary sources is inversely correlated with death from
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.
breast, prostate, ovarian cancers and risk of lung cancer (34–37). An association between low intake of Mg and the risk of colon cancer is indicated by several epidemiological studies conducted in various countries (9,38–40). Furthermore, a significant inverse correlation between dietary intake of Mg and colon cancer in men was revealed by a large population-based prospective study in Japan (8).

Experimental Mg deficiency using rat induces a clinical inflammatory syndrome characterized by leukocyte and macrophage activation, release of inflammatory cytokines and acute phase proteins, excessive production of free radicals (17). A low Mg status has been clearly associated with increased inflammatory stress in humans (41). Inflammation promotes not only in the early stages of tumorigenesis by inducing genetic instability but also in the late events, invasion and metastasis through inflammatory mediators induction (15). In the current study, we observed cancer chemopreventive activity of organo-Mg in carcinogenesis in the inflamed colon of mice. In addition, treatment with organo-Mg in the drinking water lowered the occurrence of mucosal ulcers and preneoplasms, high-grade dysplastic crypts. Interestingly, we observed that organo-Mg in the drinking water lowered the expression of proinflammatory chemokines in the colonic mucosa without tumors. It is reported that the anti-inflammatory action of Mg is caused by its ability to inactive lipopolysaccharides and to inhibit expression of several proinflammatory cytokines such as TNF-α, IL-6 and NF-κB in human placental cell, monocyte and murine macrophage-like RAW264.7 cells (42–44). In Mg-deficient mice, higher expressions of TNF-α and IL-6 have not always been observed in colonic mucosa (45). This may be caused by alterations in intestinal bifidobacteria levels. Further studies are needed to assess the role of Mg in intestinal microflora in colitis-associated colorectal carcinogenesis model that was used in this study (46).

Another interesting findings regarding mRNA expression of several proteins are that organo-Mg could increase Nr2f2 mRNA expression. Increased susceptibility to colitis-associated CRC (21) and aberrant crypt foci (23) was reported in Nr2f2-deficient mice. On the other hand, activation of Nr2f2 signaling resulted in suppression of colitis-associated CRC (22,47–49). It may be possible that increased mRNA expression of Nr2f2 by organo-Mg treatment in the inflamed colonic mucosa contributes suppressive effects of organo-Mg on inflammation-associated colorectal carcinogenesis. Additional studies are planned in our laboratory to confirm our results on mRNA expression of Nr2f2 in different experimental models of colorectal carcinogenesis with and without inflammatory stimuli.

We can point other mechanisms by which organo-Mg may suppress AOM/DSS-induced colon carcinogenesis in this study. Organo-Mg lowered MCM2-positive index and MI of colonic adenocarcinomas may suggest antigrowth effects of organo-Mg on colonic malignancy. Binding of Mg ions to DNA reduces locally the negative charge density and changes the protection pattern of the DNA from hydroxyl radicals (50). Mg is highly required to maintain genomic stability and to remove of DNA damage generated by environmental mutagens, endogenous processes and for DNA replication as an essential cofactor for several enzyme systems involving DNA repair such as nucleotide excision repair, base excision repair and mismatch repair (51). Our data on the effects of organo-Mg on the ABI of adenocarcinoma cells may suggest that organo-Mg is able to affect the CIN of cancer cells (52). In our in vitro experiment in this study, addition of a high dose (1000 µg/ml) of organo-Mg to the culture medium resulted in slight inhibition of the growth of human colorectal adenocarcinoma cell line, DLD-1 (15% inhibition, P < 0.01). However, the dose (1000 µg/ml) was quite high, suggesting cytotoxic effects of organo-Mg. We therefore should evaluate potential antigrowth effects of organo-Mg on another human colorectal cancer cell lines.

In conclusion, organo-Mg in the drinking water effectively suppressed AOM/DSS-induced mouse colon carcinogenesis by lowering proliferation and CIN in colonic adenocarcinomas in conjunction with by suppressing mRNA expression of several proinflammatory cytokines (IL-1β, IL-6, IFN-γ) and an inducible inflammatory enzyme (iNOS) and activating Nr2f2 mRNA expression. Additional in-depth studies on the effects of organo-Mg on the intestinal microflora in the colitis-associated colorectal carcinogenesis model are needed to gain further understanding of the mode of action of organo-Mg in inflammation-related carcinogenesis and to develop novel approaches for prevention of CRC in inflamed colon.

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**References**


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