Coenzyme Q10 Attenuated DMH-Induced Precancerous Lesions in SD Rats

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Summary Coenzyme Q10 (CoQ10) is known to be a compound with mitochondrial bioenergetic functions and antioxidant activity. In this study, we evaluated the effect of CoQ10 on the formation of aberrant crypt foci (ACF) induced by 1,2-dimethylhydrazine (DMH), DMH-induced leukocytic DNA damage and gene expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) by real-time PCR in colonic mucosa of male SD rats. The animals were divided into three groups and fed a casein-based high-fat and low fiber diet (100 g lard/1100 g diet) with or without CoQ10 (0.4 mg in soybean oil/kg BW/d, i.p.). One week after beginning the diets, the rats were subjected to 6 wk of treatment with DMH (30 mg/kg/wk, s.c.) and CoQ10 treatments continued over the entirety of the experimental period (59 d). Administration of CoQ10 resulted in reduction of ACF numbers, to 20% of the carcinogen control value. CoQ10 supplementation induced an antigenotoxic effect on DMH-induced DNA damage in the blood cells. Colonic mucosa of DMH-injected rats had significantly greater COX-2 and iNOS gene expression than those of control rats, while treatment with CoQ10 induced an inhibitory effect on over-expression of COX-2 and iNOS in colon tumors. Our results provide evidence that CoQ10 has a protective effect on the process of colon carcinogenesis, suppressing the development of preneoplastic lesions, possibly by modulating COX-2 and iNOS gene expression in colonic mucosa and DNA damage in leukocytes, suggesting that CoQ10 has chemotherapeutic activity.

Key Words coenzyme Q10, colon carcinogenesis, antioxidant, inflammation, DNA damage

Colon cancer is one of the leading causes of morbidity and mortality, not only in Western industrialized countries but also in developed Asian countries. The mortality rate of colon cancer increased dramatically during last 20 y while the mortality rate of cancer in other sites (stomach and liver) decreased in Korea (1). This suggests that a strong linkage may exist between colon cancer and changes in the Asian lifestyle, most particularly diet (2). A great deal of attention has become focused on the reduction of colon cancer risk via dietary modification, particularly in terms of increasing consumption of antioxidant-rich foods (fruit, vegetables, tea) (3, 4).

Coenzyme Q10 (CoQ10, also termed ubiquinone and ubidecarenone) is a fat-soluble and vitamin like compound that has an important role in the mitochondrial respiratory chain and functions as a pivotal intracellular antioxidant (5–7). CoQ10 can protect phospholipids and mitochondrial membrane proteins from peroxidation and protect DNA against oxidative damage (8–10). In addition to its direct role as an antioxidant, CoQ10 can regenerate other antioxidants such as ascorbic acid and a-tocopherol (5–7).

During carcinogenesis in the colon, aberrant crypts can be recognized as early neoplastic lesions in both rodents and humans (11). A number of natural chemopreventive agents or medicinal plants which inhibit the development of aberrant crypt foci (ACF) have been demonstrated to prevent colon cancer in rodents (12, 13), thereby suggesting that ACF assays in the rodent colon can be employed as good biomarkers in colon carcinogenesis. Colon carcinogenesis is also known to be a pathological consequence of persistent oxidative stress, resulting in DNA damage and mutations in cancer-associated genes, cycle arrest or cell death, and regeneration in which the cellular overproduction of reactive oxygen molecules and reactive nitrogen species have been implicated (14–17).

Recently, Sakano et al. have reported that the supplementation of CoQ10 resulted in a significant reduction of ACF numbers in azoxymethane (AOM) induced colonic carcinogenesis in F344 rats (18). However the mechanism of this protection has not been fully elucidated.

Therefore, we investigated the effects of CoQ10 on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis via the DNA damage and gene expression related to inflammation with the modulation of ACF in SD rats.

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MATERIALS AND METHODS

Animal and diets. Five-week-old male Sprague-Dawley (SD) rats (185 ± 10 g) were purchased from Koatec Inc. (Osan, Korea) and housed individually in hanging wire cages in a room controlled for humidity (55%), temperature (25˚C) and a 12/12 h light-dark cycle. They were cared for in accordance with the Guide for Care and Use of Laboratory Animals (Department of Health, Education, and Welfare, 1985) and the experimental protocols were approved by the Institutional Ethics Review Committee for Animal Experimentation. The rats were allowed free access to water and food for the first week with a commercially prepared pelleted diet for adjustment. The SD rats (185 ± 10 g) were divided into 3 groups of 10 animals each, and fed on a high-fat and low-fiber diet as the diet of some western populations at risk for colon cancer according to Femia et al. (19). The normal control group (NC) and positive control group (DMH) received a high-fat and low-fiber diet and CoQ10 (Sigma, 0.4 mg/kg BW, i.p. once daily) injection; the CoQ10 supplemented group (DMH/CoQ) received a high-fat and low-fiber diet and CoQ10 (Sigma, 0.4 mg in soybean oil/kg BW, i.p. once daily) injection. One week after beginning the diets, rats were treated with 1,2-dimethylhydrazine (DMH, Fluka, St. Louis, MO, USA). 30 mg/kg, s.c. once a week) for 6 wk except for the normal control group (NC), which was treated with saline instead; CoQ10 treatments continued for the entire experiment (59 d). The composition of the diet was starch (51%), casein (20%), corn oil (10%), lard (12%), cellulose (2%), mineral and vitamin mixture (3.5 and 1%, AIN-93; American Institute of Nutrition, 1993), choline bitartrate (0.25%), cellulose (2%), mineral and vitamin mixture was starch (51%), casein (20%), corn oil (10%), lard (12%), mineral and vitamin mixture.

The segment was washed with physiological saline (0.9% NaCl), cut open, and fixed in 10% buffered formalin solution for at least 24 h. Later, the colon samples were stained with 0.2% methylene blue for 5 min, and the mucosal side was placed on a glass slide and examined microscopically using ×10 objective for assessment of the number of aberrant crypts (AC) following a procedure described by Bird (11).

DNA damage determination by alkaline comet assay. The alkaline comet assay was conducted according to the protocols established by Singh et al. with little modification (20). Frosted slides (Fisher Scientific) were prepared with a basal layer of 0.5% normal melting agarose; then 5 μL of whole blood, mixed with 75 μL of 0.7% low melting agarose (LMA), was added to the slides. The slides were again covered with coverslips, and kept in the refrigerator for 10 min. The coverslips were then removed, and a top layer of 75 μL 0.7% LMA was added before placing the slides (with coverslips) in the refrigerator again for 10 min. After removal of the coverslips, the slides were immersed in a jar containing pH 10 cold lysing solution, consisting of 2.5 mol NaCl, 100 mmol/L EDTA, 10 mmol/L Tris, and 1% sodium laurylsarcosine; 1% Triton X-100 and 10% DMSO were freshly added to the solution, which was then stored in the refrigerator for 1 h. After lysis, the slides were placed in a horizontal electrophoresis tank (Threeshine Co., Ltd., Korea). The slides were covered with a fresh alkaline buffer (300 mmol/L NaOH, 10 mmol/L Na2EDTA, pH 13.0), and maintained at 4˚C for 40 min. Electrophoresis of the DNA was accomplished by applying an electric current of 25 V/300 ± 3 mA for 20 min at 4˚C. The slides were washed three times with neutralizing buffer (0.4 mol Tris, pH 7.5), for 5 min at 4˚C, and then were treated with ethanol for another 5 min. All steps following the lysis treatment were undertaken in darkness in order to prevent additional DNA damage. Fifty microliters of ethidium bromide (20 μg/mL) was added to each slide, which was then analyzed using a fluorescence microscope (LEICA DMLB, Bensheim, Germany). Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed from each subject. Measurements were made by image analysis (Kinetic Imaging, Komet 4.0, Liverpool, UK), determining percentage of DNA in the tail and tail moment (TM, calculated as the percentage of DNA in the tail times tail length).

Real-time quantitative PCR. Total RNA was isolated from the mucosa in the distal part of the colon using the.

Table 1. Primer sequences used for real-time PCR amplification.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequences (in 5’–3’ direction)</th>
<th>Base pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>sense: ACCAACGCTGCCACAACATG</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>antisense: GGTGGAAACGCAAGGATTT</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>sense: ACCATGGAGCATCCCAAGTGA</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>antisense: CAGGCGCATACCTCCAGGC</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>sense: CCCCAGATACAACCTTCT</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>antisense: GTACATTCCAGGCGGAAC</td>
<td></td>
</tr>
</tbody>
</table>

COX-2: cyclooxygenase-2, iNOS: inducible nitric oxide synthase.
Trizol protocol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed using the Superscript First-Strand cDNA Synthesis kit (Invitrogen). Primer sequences and sizes of PCR amplification products are described in Table 1. Thermal cycling conditions for the PCR reactions were 95°C for 10 min followed by 55 cycles of 95°C for 15 s, 55°C for 20 s, and 72°C for 20 s. Real-time PCR reactions were carried out with an iCycler real-time machine (Biorad, Hercules, CA, USA) using the IQ SYBR Green Supermix kit (Biorad). The levels of mRNA for each gene were normalized against β-actin.

Statistical analysis. Data were analyzed using the SPSS package for Windows (Version 10). Values were expressed as mean ± standard error (SE). The data was evaluated by one-way ANOVA and the differences between the means assessed using the Tukey test. Student’s t-test was employed to compare the DMH group with the CoQ10 supplement group. The differences were considered significant at p < 0.05.

RESULTS

Food intake, weight gain, and organ weight

No treatment-associated signs of adverse effects in the clinical appearance of the animals were noted during this experiment. No differences were detected among the three groups with regard to food consumption or body weight (data not shown).

Colon aberrant crypt foci formation

All rats injected with DMH developed abnormal and hyperplastic crypts in the colon, while no abnormal or hyperplastic crypts were found in the rats treated with saline (Fig. 1). Aberrant crypts (AC) are wider than normal crypts and exhibit a slit-like opening when viewed from above compared to the circular appearance of normal crypts. The lesions can be classified as single enlarged ACs or foci containing two or more abnormal ACs with thickened epithelial linings and enlarged luminal openings. A significant reduction of 80% (129.7 ± 42.0 vs. 27.3 ± 10.7, p < 0.05) was noted in the average total number of ACF per colon, as well as the numbers of foci harboring one and two crypts in the 1.2-DMH-treated rats supplemented with CoQ10, in comparison to what was observed in the rats treated with the carcinogen alone (Fig. 2). The total numbers of AC in the colon were significantly reduced by 80% in the CoQ10 supplemented group (68.9 ± 23.4) as compared to the DMH group (337.1 ± 72.3) (Fig. 2).

Leukocytic DNA damage

The data in Fig. 3 demonstrate the effects of CoQ10 supplementation on DMH-induced DNA damage, expressed as tail intensity and tail length in rat peripheral blood cells. DMH treatment induced significant increases in leukocytic DNA damage, as compared with the saline-injected control group. This strong genotoxic effect of DMH was reduced significantly, by 11% in tail intensity and 28% in tail moment, after 59 d of supplementation with 3 mg of CoQ10. The comet images of the leukocytes following NC, DMH, and DMH + CoQ treatment are shown in Fig. 4.

![Fig. 1. Aberrant crypt foci. A: Normal colon without any ACF, rats were treated with saline. B: Colon with 3 aberrant crypts/ACF, rat was treated with DMH (carcinogen of colon).](image)

![Fig. 2. Effect of CoQ10 treatment on total number of aberrant crypt foci (ACF) and total number of aberrant crypts (AC) induced by DMH in the distal colon. Bars represent mean ± SE for 10 animals in each group. Significantly different from the DMH group: **p < 0.01 (Student’s t-test).](image)
Real-time quantitative PCR

The expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was evaluated in colon mucosa (Table 2). The colon mucosa of DMH-treated rats had significantly greater COX-2 and iNOS gene expression than the mucosa of saline-treated rats, while treatment with CoQ10 induced an inhibitory effect on over-expression of COX-2 and iNOS in colon mucosa.

**DISCUSSION**

The aim of this study was to investigate the effects of Coenzyme Q10 (CoQ10) on 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis via the DNA damage and gene expression of COX-2 and iNOS with the modulation of ACF in SD rats. Injection of CoQ10 (0.4 mg in soybean oil/kg BW/d, i.p.) for 59 d effectively suppresses the occurrence of colonic ACF induced by DMH when administered 1 wk prior to treatment with the carcinogen. The colonic carcinogen, DMH, induces a high incidence of pre-cancerous lesions, which are referred to as ACF, in rats. ACF, which are enlarged and elevated relative to normal crypts, have also been detected in the human colon, and are generally associated with carcinoma (21, 22). Owing to the potential progression of early changes to malignancy, the study of premalignant hyperproliferative lesions and aberrant crypts is crucial to our understanding of colon cancer pathogenesis (23). Similar results have been observed in the colons of rats treated with CoQ10 administered during the post-initiation phase of DMH or AOM-induced carcinogenesis (18, 24). Suzuki et al. have found that rats administered the colon carcinogen, DMH, and fed on diets with 0.2 mg/d per rat of CoQ10 for 23 wk evidenced a 42% and 46% reduction in the number of invasive carcinomas and the number of lesions with epithelial dysplasia per rat, relative to a control diet containing no CoQ10 (24). Recently, Sakano et al. have reported that the supplementation of CoQ10 at a dose of 2.5 and 6.3 mg/d per rat for 4 wk resulted in reduction of ACF numbers to 77% and 68% of the control value, respectively, in azoxymethane (AOM)-induced colonic carcinogenesis in F344 rats (18). Note that, in that study, although we supplied a lower concentration of CoQ10 (average injection of CoQ10 throughout the study period was 0.15 mg/d per rat, when divided by the average body weight) than did Sakano et al. (18), the ACF lowering effect was higher in our study. This can be explained either by the different route of admin-

**Table 2. Effect of CoQ10 on DMH-induced gene expression in colon mucosa.**

<table>
<thead>
<tr>
<th></th>
<th>NC</th>
<th>DMH</th>
<th>DMH + CoQ</th>
</tr>
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<tbody>
<tr>
<td>COX-2</td>
<td>0.10 ± 0.08a</td>
<td>2.23 ± 1.07b</td>
<td>0.53 ± 0.26a</td>
</tr>
<tr>
<td>iNOS</td>
<td>0.61 ± 0.29a</td>
<td>2.23 ± 0.88b</td>
<td>0.84 ± 0.45a</td>
</tr>
</tbody>
</table>

1Data represent mean ± SE for 10 animals in each group. a,b Values with different superscripts are significantly different at the p < 0.05 level by the Tukey test. The levels of mRNA for each gene were normalized against β-actin.

**Fig. 3.** Effect of CoQ10 treatment on DMH-induced DNA damage in leukocytes. Bars represent mean ± SE for 10 animals in each group. Significantly different from the DMH group: Bars with different superscripts are significantly different at the p < 0.05 level by the Tukey test.

**Fig. 4.** Fluorescence comet image of rat leukocytes dyed with ethidium bromide (×400).

**Fig. 3.** Effect of CoQ10 treatment on DMH-induced DNA damage in leukocytes. Bars represent mean ± SE for 10 animals in each group. Significantly different from the DMH group: Bars with different superscripts are significantly different at the p < 0.05 level by the Tukey test.
istration (i.p. injection vs. oral feeding), colon carcinogen (DMH vs. AOM), experimental duration (15 wk vs. 4 wk), or strain of rats (SD vs. F344 rat). Although there were some differences in those variables across the studies, the common point is that rats received CoQ10 before the first dose of colon carcinogen so that the prevention effects might be partly attributable to inhibition of initiation by colon carcinogens. The mechanism underlying the preventive effect of CoQ10 in our study may be related to a reduction in COX-2 and iNOS mRNA expression in colon mucosa. COX-2 and iNOS overexpression has been demonstrated in human cancer cells and in carcinogen induced rat colonic tumors (25–27), a phenomenon which has been related to inflammation, resistance to apoptosis, DNA damage, mutation, increased proliferation, and oxidative stress (28). It is also reported that inhibition of COX-2 and iNOS expressions decrease DMH-induced carcinogenesis (29). The molecular mechanisms by which CoQ10 mediates anticarcinogenic effects are uncertain. To our knowledge, this is the first study to report a significant decrease in COX-2 and iNOS mRNA expression in DMH-treated colon mucosa upon CoQ10 treatment. Oztay et al. have found that CoQ10 administration (1.5 mg/kg BW for 2 wk) decreased iNOS immunoreactivities in heart tissue in hyperthyroidism Swiss Black C57 mice receiving 5 mg/kg L-thyroxine (30). The expression of iNOS was reduced by quercetin without a statistical significance, and COX-2 expression was slightly reduced by beta-carotene supplementation in colonic mucosa of rats treated with AOM, along with a decreased number of ACF (31).

DMH has been reported to induce carcinogenesis in rodents via high levels of reactive free radical production, decreased activity of some antioxidant enzymes, and increased DNA damage (32, 33). As DNA damage has been implicated as the initial step in chemical carcinogenesis, the blocking of DNA damage should constitute the first line of defense against cancer induced by carcinogens (34). Our results indicated that CoQ10 treatment effectively attenuated the genotoxic effects of DMH in leukocytes. Since CoQ10 exhibits strong antioxidant activity (5–10), this property could be classified as a putative chemopreventive agent against oxidative DNA damage (35). Similarly to our result, Tomasetti et al. have found that in vitro CoQ10 supplementation enhanced DNA resistance towards H2O2-induced oxidative DNA damage in human lymphocytes (10). Nikłowitz et al. confirmed that oral CoQ10 supplementation supported in vivo intracellular antioxidative defense mechanisms, as inhibition of DNA strand break down in lymphocytes was demonstrated (36).

In summary, the data presented herein suggest that CoQ10 treatment reduced colonic precarcinogenic events in rats in which colon carcinogenesis had been induced with DMH, and that this effect might be attributed to a modulation of COX-2 and iNOS gene expression and antigenotoxic property. The results of this study provide us with new insight into the mechanisms underlying the anticancer properties of CoQ10. Further studies will be needed to understand the role of CoQ10 in gene-expression during colon carcinogenesis.

Acknowledgments
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REFERENCES
16) Nair S, Norkus EP, Hertan H, Pitchumoni CS. 2001. Serum and colon mucosa micronutrient antioxidants: differences between adenomatous polyp patients and


