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NZ-419, a Radical Scavenger, Inhibits Intestinal Polyp Formation in Apc-Mutant Mice

Makoto Hosoya[1,2], Natsuki Kawashima[3*], Shinichi Negishi[4]

[1] Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan.
[2] Laboratory of Molecular Pathology and Metabolic Disease, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba 278-8510, Japan.
[3] Central Radioisotope Division, National Cancer Center Research, Tokyo 104-0045, Japan.
[4] Department of Microbiology and Immunology, Aichi Medical University, Nagakute, Aichi 480-1195, Japan.

Abstract

Colorectal cancer ranks as the fourth most common cause of cancer-related mortality worldwide, making the development of preventive strategies a critical priority. Antioxidants have been considered as potential preventive agents; however, the contribution of their direct radical-scavenging activity to cancer prevention remains uncertain. In this study, we investigated whether NZ-419, a hydroxyl radical scavenger, could suppress colorectal tumorigenesis through direct elimination of reactive oxygen species (ROS). NZ-419, a metabolite of creatinine with proven safety and prior efficacy in delaying chronic kidney disease progression in rats, is currently under clinical evaluation. Our findings revealed that NZ-419 significantly reduced ROS production in HCT116 cells exposed to H_2O_2 and downregulated H_2O_2-induced transcriptional activation of the Nrf2 promoter. Oral supplementation of 500 ppm NZ-419 to Apc-mutant Min mice for 8 weeks resulted in a marked reduction of small intestinal polyps, particularly in the middle region, lowering polyp numbers to 62.4% of those in untreated controls ($p < 0.05$). As anticipated, NZ-419 treatment altered serum levels of reactive carbonyl species, serving as oxidative stress indicators. Furthermore, polyp tissues from treated mice exhibited decreased expression of the proliferation-related oncogene c-Myc, accompanied by modest suppression of epithelial cell proliferation assessed via PCNA staining. Collectively, these results indicate that NZ-419 inhibits intestinal polyp formation in Min mice, highlighting the potential of radical scavenger-based antioxidants as chemopreventive agents against colorectal cancer.

Keywords: Apc-mutant mice, Cancer chemoprevention, NZ-419, Nrf2, Hydroxyl radical

Corresponding author: Natsuki Kawashima
E-mail: Natsukikawashima@outlook.com

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Introduction

Colorectal cancer (CRC) remains a major global health burden, accounting for nearly 8% of all cancer-related deaths and ranking as the fourth leading cause of cancer mortality worldwide [1]. This high incidence underscores the urgent need to establish effective preventive strategies. Among the proposed approaches, chemoprevention through the use of antioxidants has received considerable attention.

Despite this promise, significant uncertainties remain. Numerous epidemiological, clinical, and preclinical investigations suggest that lowering oxidative stress could reduce CRC risk. However, antioxidants often exert their anticancer properties not only through direct radical scavenging but also via diverse biological mechanisms. For example, ω3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA, C22:6) and eicosapentaenoic acid (EPA, C20:4)—key constituents of fish oil—have been widely tested in rodent colon cancer models [2]. Clinical validation was provided by a phase III randomized, double-blind, placebo-controlled trial, in which familial adenomatous polyposis (FAP) patients treated with EPA (2 g/day for 6 months) exhibited a 22.4% reduction in polyp numbers ($p = 0.012$) and a 29.8% reduction in total polyp diameters ($p = 0.027$) compared with placebo [3]. While PUFAs are prone to peroxidation and can lower reactive oxygen species (ROS) levels, they also inhibit cyclooxygenase (COX) enzymes and function as ligands for G protein–coupled receptors (GPCRs) [2,3]. Similarly, lutein, a carotenoid with strong radical-scavenging activity, has been reported to regulate cell proliferation [4,5] and apoptosis signaling [6] in addition to its antioxidant effects. Many other phytochemicals also demonstrate dual antioxidant and anticarcinogenic properties [7–10]. These findings collectively suggest that antioxidants are promising candidates for chemoprevention in CRC, although the extent to which their antioxidative capacity alone drives this protection remains unresolved.

To clarify this question, direct evidence is needed to show whether ROS elimination itself can suppress colorectal carcinogenesis. Prior research demonstrated that mesalamine (5-aminosalicylic acid, 5-ASA) directly scavenges peroxynitrite and significantly inhibits DNA strand breaks caused by the peroxynitrite donor 3-morpholinosydnonimine at concentrations of 0.1–1.0 mM [11]. Moreover, 5-ASA use has been linked to a reduced risk of CRC in patients with ulcerative colitis. Nevertheless, in animal studies using Min mice (FAP model mice harboring an Apc mutation that predisposes them to intestinal polyps), dietary administration of 5-ASA at doses ranging from 500 to 9600 ppm did not demonstrate clear chemopreventive activity [12].

In pursuit of a more effective orally available ROS scavenger, we identified 5-hydroxy-1-methylhydantoin (NZ-419; **Figure 1A**), a metabolite of creatinine, which exhibits hydroxyl radical (·OH) scavenging properties. NZ-419 has previously been shown to slow the progression of chronic kidney disease in rat models [13,14]. Given that creatinine itself is an endogenous ·OH scavenger and its metabolites are characterized by low toxicity, NZ-419 emerges as a promising candidate. Importantly, this compound is now undergoing clinical development as a potential therapy for advanced chronic renal failure (stages 3–4 of chronic kidney disease) and is currently in Phase II clinical trials.

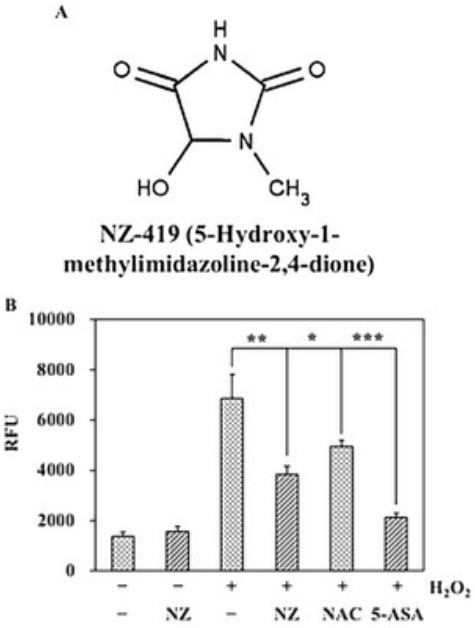


Figure 1. Suppression of reactive oxygen species (ROS) formation in HCT116 cells by NZ-419. (A) Structural formula of NZ-419. (B) HCT116 cells were pre-incubated for 30 minutes with either 1 mM NZ-419 (NZ), 5 mM N-acetyl-L-cysteine (NAC), or 100 μM 5-aminosalicylic acid (5-ASA). Afterward, the cells were challenged with 0.5 mM hydrogen peroxide and maintained for an additional 10 minutes to monitor ROS levels. NAC and 5-ASA served as positive reference compounds. Data are expressed as mean values ± SD ($n = 3$). Significant differences compared with untreated controls are marked as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.005$. The figure represents typical outcomes confirmed across more than three independent trials

This experiment verified that NZ-419 acts as a hydroxyl radical (·OH) scavenger in human colorectal cancer cells. We next assessed whether its administration could reduce intestinal polyp burden in Min mice.

Materials and Methods

Chemicals

NZ-419 was provided by Nippon Zoki Pharmaceutical Co., Ltd. Hydrogen peroxide (H_2O_2) was sourced from Wako Pure Chemical Industries (Osaka, Japan). Sigma-Aldrich (St. Louis, MO, USA) supplied N-acetyl-L-cysteine, acetaldehyde, p-toluenesulfonic acid (p-TsOH), and a panel of reactive carbonyl compounds (RCs), including propanal, pentanal, butanal, 2-hexenal, hexanal, 2-heptenal, heptanal, octanal, 2-nonenal, nonanal, decanal,

undecanal, dodecanal, and tridecanal. 5-aminosalicylic acid was obtained from Tokyo Chemical Industry (Tokyo, Japan), which also supplied acrolein, crotonaldehyde, dansyl hydrazine (DH), glyoxal, 2,4-decadienal (DDE), heptadecanal, hexadecanal, 2,4-nonadienal (NDE), octadecanal, 2-octenal, pentadecanal, tetradecanal, and 2-undecenal. Cayman Chemical Company (Ann Arbor, MI, USA) provided 4-hydroxy-2-hexenal (HHE), 4-hydroxy-2-nonenal (HNE), and 4-oxo-2-nonenal (ONE). Wako Pure Chemical Industries supplied p-benzyloxybenzaldehyde (p-BOBA). Unusual aldehydes such as 8-heptadecenal (8-HpDE), 8,11-heptadecadienal (8,11-HpDDE), and 8,11,14-heptadecatrienal (8,11,14-HpDTE) were synthesized according to established procedures [15,16], while secosterol-A and secosterol-B were prepared following Wentworth *et al.* [17]. Stock solutions of all RCs and the internal standard p-BOBA (10 μM) were dissolved separately in acetonitrile and kept at −20 °C until required.

Cell Culture

Human colorectal carcinoma HCT116 cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories Inc., Logan, UT, USA) and antibiotics (100 μg/mL streptomycin and 100 U/mL penicillin). Cultures were grown at 37 °C in a humidified incubator with 5% CO_2.

Animals

Male C57BL/6-ApcMin/+ mice (Min mice) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Groups of three to four animals were housed in sterilized plastic cages lined with softwood bedding under controlled conditions (24 ± 2 °C, 55% relative humidity, 12 h light/dark cycle). The basal diet AIN-76A (Japan CLEA, Tokyo, Japan) was supplemented with NZ-419 at concentrations of 500 or 1000 ppm, and the diets were refreshed every two weeks.

Animal experimental protocol

Nine male Min mice, 5 weeks of age, were divided into three groups and provided diets containing 0, 500, or 1000 ppm NZ-419 for a period of 8 weeks. Each cage housed animals from the same treatment group. Food and water were given without restriction. A specialized hanging bait box was used to prevent spillage or contamination of the diet with urine, and this system also allowed accurate measurement of food intake per cage. The animals were checked daily for health status and survival, while body weight and diet consumption were recorded on a weekly basis.

At the endpoint, mice were anesthetized, and blood samples were collected via the abdominal vein. The gastrointestinal tract was excised and divided into the small intestine, cecum, and colon. The proximal 4 cm of the small intestine was isolated, and the remainder was split evenly into middle and distal sections. Polyps in the proximal region were counted and excised under a stereomicroscope at necropsy. Non-polyp mucosal tissue from this segment was scraped, snap-frozen, and stored at −80 °C for subsequent quantitative RT-PCR. Other intestinal portions were opened longitudinally, placed flat between filter papers, and fixed in 10% neutral-buffered formalin. Polyp number, diameter, and distribution were then evaluated microscopically. All procedures adhered to the Guidelines for Animal Experiments in the National Cancer Center and were approved by the institutional animal ethics committee. Every effort was made to minimize animal discomfort. Mice were acclimated for at least one week before the experiment, and all were euthanized by isoflurane overdose prior to tissue collection.

Measurement of intracellular ROS

HCT116 cells (2×10^4 per well) were seeded in black 96-well plates (Sigma-Aldrich) and cultured for 24 h. Intracellular ROS was assessed using 1 mM DCFH-DA (Cell Biolabs, San Diego, CA, USA), incubated with cells for 60 min at 37 °C in the dark. Cells were washed three times with PBS, then treated with either 1 mM NZ-419, 5 mM NAC, or 100 μM 5-ASA for 30 min. Afterward, they were challenged with 0.5 mM H_2O_2 for 10 min at 37 °C. Fluorescence intensity was measured on an iMark™ Microplate Absorbance Reader (Bio-Rad, Hercules, CA, USA) at 485 nm excitation and 535 nm emission. Data represent mean ± SD (n = 3).

Luciferase reporter assay for Nrf2 transcriptional activity

To evaluate Nrf2 promoter activity, HCT116 human colon carcinoma cells were transfected with the pNrf2/ARE-Luc plasmid (Promega, Madison, WI, USA) using polyethylenimine MAX (MW 40,000; PolyScience, Warrington, PA, USA). Stably transfected clones, selected under hygromycin pressure, were designated HCT116-Nrf2-Luc cells. These cells were plated in 96-well plates (2×10^4 per well) and cultured for 24 h. They were then treated with NZ-419 at concentrations of 1, 10, 100, or 10,000 μM, either alone or in combination with 40 μM H_2O_2, for 24 h. Firefly luciferase activity was quantified using the Bright-GLO assay system (Promega). Control basal activity was normalized to 1.0. Results are presented as mean ± SD (n = 3).

Extraction and profiling of reactive carbonyl species (RCs)

Mouse serum was homogenized in sodium phosphate buffer (50 mM, pH 7.4) containing 0.5 mM EDTA and 20 µM BHT. Each sample was spiked with the internal standard p-BOBA (20 pmol) and extracted with 400 µL of chloroform/methanol (2:1, v/v). After 1 min of vortexing, samples were centrifuged at 15,000 rpm for 10 min, and the organic fraction was collected. The remaining phases were re-extracted with the same solvent mixture, centrifuged again, and combined with the first extract. To derivatize RCs, the pooled organic fraction was incubated with 100 µL acetonitrile containing 50 µg DH and 10 µg p-TsOH for 4 h at room temperature in the dark. After evaporation under vacuum, the residues were dissolved in 200 µL acetonitrile, and 5 µL aliquots were analyzed by LC/MS.

Chromatographic separation of RC–DH adducts was achieved on a CORTECS UPLC C18 column (1.6 µm, 100 × 2.1 mm; Waters) using a Shimadzu Nexera X2 UHPLC system coupled to a Sciex TripleTOF 5600+ mass spectrometer equipped with a Duospray™ source. The mobile phases consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Gradient conditions were: 20% B at 0 min, ramp to 100% B at 5 min, hold until 12 min, then return to 20% B at 12.01 min, followed by 3 min re-equilibration. Flow rate was set at 0.4 mL/min. MS acquisition in positive ion mode was performed with the following parameters: curtain gas, 35 psi; ionspray voltage, 5500 V; source temperature, 350 °C; ion source gases 1 and 2, 60 psi each; declustering potential, 80 V; collision energy, 45 V with a 15 V spread. Detection of RC–DH derivatives was carried out using MRMHR mode. The analytical workflow was adapted from previously described protocols [18, 19].

Quantitative Real-Time PCR (qPCR)

Total RNA was extracted with TRIzol reagent (Invitrogen, Grand Island, NY, USA). For cDNA preparation, 1 µg of RNA in a 20 µL reaction mixture was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Quantitative PCR was then performed on a Bio-Rad CFX96/384 Detection System with Fast Start Universal SYBR Green Master Mix (Roche Diagnostics, Mannheim, Germany), following the manufacturers' protocols. The primer pairs used were: c-Myc (forward 5′-GCCCGCGCCCAGACAGGATA, reverse 3′-GCGGCGGCGGAGAGGA), Cdk4 (forward 5′-CTGGTGTTTGAGCATGTAGACC, reverse 3′-GATCCTTGATCGTTTCGGCTG), and GAPDH (forward 5′-TTGTCTCCTGCGACTTCA, reverse 3′-CACCACCCTGTTGCTGTA). Amplification specificity for each set of primers was confirmed by analyzing melting curve profiles.

Immunohistochemistry

Sections of the mid-small intestine were prepared using the Swiss roll method after fixation and paraffin embedding. Immunostaining was performed with the avidin–biotin peroxidase technique. Proliferating cell nuclear antigen (PCNA) was detected using a monoclonal anti-PCNA antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution 1:200). The secondary antibody was a biotinylated anti-mouse IgG preabsorbed with horse serum (Vector Laboratories, Burlingame, CA, USA; dilution 1:200). Visualization was achieved using the Vectastain ABC system together with 3,3′-diaminobenzidine and H_2O_2, followed by hematoxylin counterstaining. Images of all intestinal polyps were captured at 20× magnification, and the proliferation index was calculated as the percentage of PCNA-positive nuclei relative to the total nuclei per polyp.

Statistical Methods

Data are reported as mean values ± standard deviation. Comparisons between groups were made using Student's t test, except for polyp counts and luciferase activity data, which were analyzed with Dunnett's test. Statistical significance thresholds were set at $p < 0.05$, $p < 0.01$, and $p < 0.005$.

Results

Reduction of ROS by NZ-419 in HCT116 Cells

The antioxidant effect of NZ-419 was assessed in HCT116 cells using the ROS-sensitive probe H_2DCFDA, which detects intracellular hydrogen peroxide and superoxide. Exposure to 0.5 mM H_2O_2 for 10 min markedly increased fluorescence, whereas pretreatment with 1 mM NZ-419 reduced the signal to 55.9% of the untreated control. In comparison, the known ROS scavengers NAC and 5-ASA reduced ROS levels to 72.1% and 31.0%, respectively **(Figure 1B)**.

Inhibition of H_2O_2-Induced Nrf2 activation by NZ-419

To examine the role of NZ-419 in regulating antioxidant signaling, its effect on nuclear factor erythroid 2–related factor 2 (Nrf2) promoter activity was tested. Stimulation with H_2O_2 alone enhanced Nrf2-driven transcription by 354% relative to the control group ($p < 0.005$). However, when cells were treated with 10,000 µM NZ-419 for 24 h in the presence of H_2O_2, this induction was suppressed by 56% ($p < 0.01$). Importantly, NZ-419 treatment alone did not decrease the basal activity of the Nrf2 promoter **(Figures 2A, 2B)**.

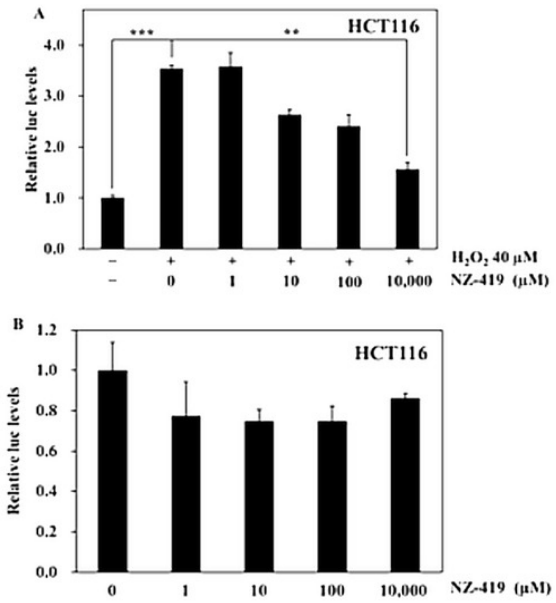


Figure 2. NZ-419 Inhibits H2O2-Stimulated Nrf2 Promoter Activity. HCT116 cells were exposed to 10,000 µM NZ-419 and incubated for 30 minutes at 37 °C. Afterward, cells were either treated with 40 µM H2O2 for 24 hours (panel A) or maintained for 24 hours without H2O2 exposure (panel B). Luciferase activity in untreated control cells was set as 1.0 for reference. Data are expressed as the mean ± SD (n = 3). Statistical significance is indicated as ** $p < 0.01$ and *** $p < 0.005$ compared to control. The findings are representative of results obtained from at least three separate experiments

NZ-419 reduces intestinal polyp development in min mice

Min mice receiving NZ-419 at concentrations of 500 or 1000 ppm for eight weeks showed no differences in food intake **(Figure 3A)**, body weight **(Figure 3B)**, or clinical condition throughout the experiment. Daily food consumption was similar across all groups, including control, 500 ppm, and 1000 ppm. Organ weights also remained unchanged, suggesting no overt toxicity. **Table 1** summarizes the polyp counts and their locations in control versus NZ-419-treated mice. Most polyps formed in the small intestine, with few appearing in the colon. Administration of 500 ppm NZ-419 led to a notable reduction in polyp numbers within the middle segment of the small intestine, reaching 62.4% of control levels ($p < 0.05$), while other intestinal regions and the colon showed no significant change. **Figure 4** presents the size distribution of polyps across all groups, revealing that the majority measured between 0.5 and 1.0 mm in diameter.

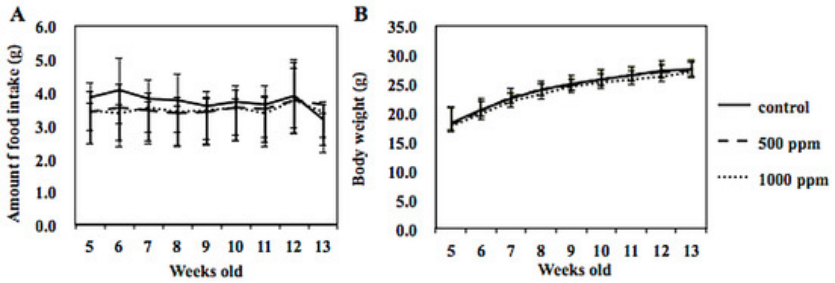


Figure 3. Weekly Monitoring of Food Consumption and Body Weight. The daily food intake per mouse (A) and the body weight of individual mice (B) were recorded on a weekly basis throughout the study period

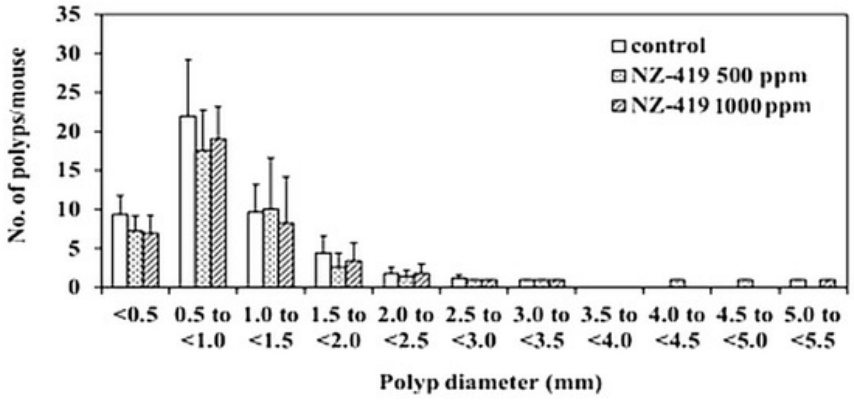


Figure 4. NZ-419 reduced intestinal polyp growth. The graph displays the distribution of polyp sizes in mice fed a standard control diet compared with those treated with NZ-419

Table 1. The number of intestinal polyps/mouse in Min mice treated with or without NZ-419

Dose (ppm)	No. of Mice	Small Intestine			Colon	Total
		Proximal	Middle	Distal		

0	9	5.8 ± 2.9	11.7 ± 3.4	29.3 ± 5.4	0.7 ± 1.0	47.4 ± 5.2
500	9	3.9 ± 1.6	7.3 ± 2.9 *	27.0 ± 10.2	1.0 ± 0.9	39.2 ± 10.3
1000	8	5.1 ± 2.4	8.3 ± 2.8	25.6 ± 7.2	0.4 ± 0.7	39.4 ± 9.6

Data are shown as mean ± standard deviation (SD). Values marked with * indicate a statistically significant difference compared to the untreated control group (* $p < 0.05$).

The levels of oxidative stress-related markers in min mice by NZ-419

To assess NZ-419's influence on oxidative stress, we measured reactive carbonyl species (RCs) in the serum of Min mice using liquid chromatography–mass spectrometry (LC/MS), comparing treated and untreated animals. Preliminary results suggested that the effect of NZ-419 plateaued at 500 ppm, so we analyzed samples from mice given 1000 ppm to maximize observable differences. LC/MS profiling revealed 509 peaks in the serum of untreated mice and 495 peaks in the NZ-419-treated group **(Figure 5A,B)**. Of these 495 peaks, 305 were lower in treated mice relative to controls, with 31 showing statistically significant reductions, including 2-Nonenal and HNE ($p < 0.05$) **(Figure 5C)**.

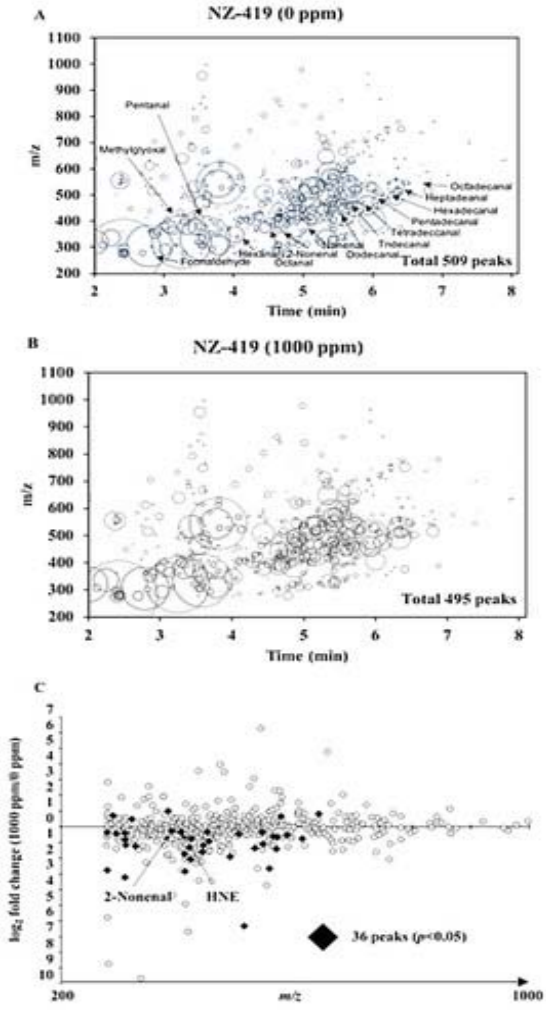
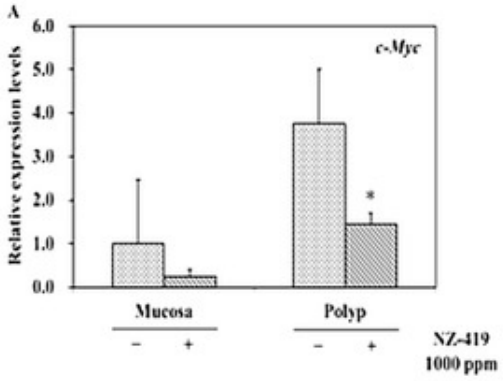


Figure 5. Maps of reactive carbonyl species (RCs) showing the free RCs detected in the serum of Min mice. Panels show RCs from mice treated with 0 ppm (A) or 1000 ppm (B) NZ-419. Each RC is represented by a circle, plotted according to its retention time (x-axis) and m/z value (y-axis), with circle size reflecting the peak intensity relative to the internal standard (IS). Abbreviations for the RCs are provided in the Materials and Methods section. (C) Comparative RC profiles in serum from mice treated with 1000 ppm NZ-419. Closed diamonds indicate RCs that differed significantly between untreated and NZ-419-treated mice ($p < 0.05$)

NZ-419 reduces mRNA expression of proliferation-associated factors in intestinal polyps of min mice

To investigate how NZ-419 affects genes regulated by Tcf/LEF or involved in cell cycle control, mRNA levels of these target genes were measured in both polyp and non-polyp regions of the intestine in Min mice. After 8 weeks of treatment with 1000 ppm NZ-419, c-Myc mRNA levels in polyp tissue were significantly decreased to 61% of the untreated control ($p < 0.05$), while in non-polyp mucosa, c-Myc expression declined to 74% of control levels, though this reduction was not statistically significant **(Figure 6A)**. Additionally, Cdk4 mRNA in non-polyp intestinal mucosa was reduced by 53% relative to untreated mice **(Figure 6B)**. In contrast, treatment with 500 ppm NZ-419 did not produce a statistically significant decrease in c-Myc or Cdk4 mRNA (data not shown).



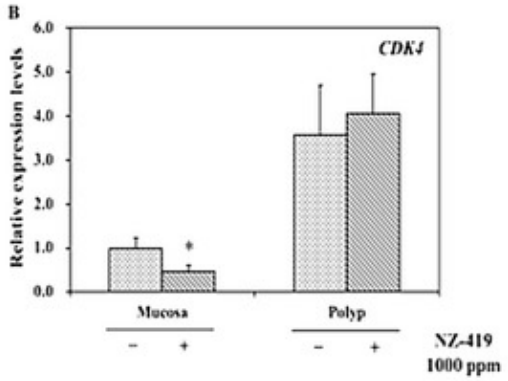


Figure 6. Inhibition of c-Myc and Cdk4 Expression in Non-Polyp and Polyp Intestinal Tissues of Min Mice Treated with 1000 ppm NZ-419. Min mice were fed diets containing NZ-419 at the indicated concentrations for 8 weeks. Quantitative real-time PCR was used to measure mRNA levels of c-Myc (A) and Cdk4 (B) in both polyp and non-polyp intestinal mucosa. Expression values were normalized to GAPDH and are presented as mean ± SD (n = 3). The control group's basal mRNA expression was defined as 1.0. * $p < 0.05$ compared with 0 ppm

NZ-419 shows a trend toward reducing cell proliferation in intestinal polyps

To evaluate the impact of NZ-419 on epithelial cell proliferation, intestinal polyps from Min mice were subjected to immunohistochemical staining using an anti-proliferating cell nuclear antigen (PCNA) antibody. As illustrated in **Figure 7**, the proliferation index (calculated as the ratio of PCNA-positive nuclei to total nuclei within the polyp) suggested that treatment with 1000 ppm NZ-419 tended to reduce cell proliferation in the intestinal polyps of Min mice.

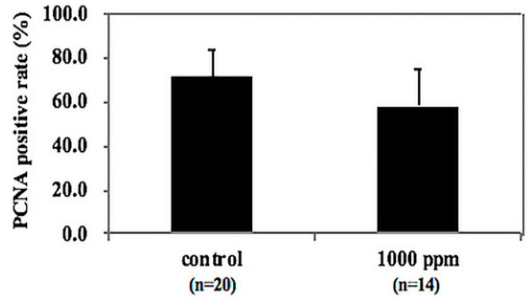


Figure 7. NZ-419 Reduces Epithelial Cell Proliferation. The effect of NZ-419 on cell proliferation in intestinal polyps was assessed by measuring the proportion of PCNA-positive cells. Immunohistochemical staining for PCNA was performed on intestinal polyps from Min mice fed either a basal diet or a diet containing 1000 ppm NZ-419. The method for calculating the PCNA-positive cell ratio is detailed in the Materials and Methods section. Bars represent SD (n = 7–11)

Discussion

In this study, we demonstrated that NZ-419 effectively suppressed ROS generation in HCT116 cells following H2O2 stimulation and inhibited H2O2-induced transcriptional activity of the Nrf2 promoter in vitro. Notably, NZ-419 did not affect Nrf2 transcriptional activity in the absence of H2O2, suggesting that its actions may be at least partially dependent on ROS. In vivo, NZ-419 treatment reduced intestinal polyp formation in Min mice. While mRNA levels of the proliferation-associated gene c-Myc were decreased in intestinal polyps after NZ-419 administration, immunohistochemical analysis using PCNA staining revealed only a modest suppression of cell proliferation in these polyps.

Our findings align with previous reports demonstrating the ·OH scavenging capacity of NZ-419. To evaluate this, we employed H2DCFDA, an oxidation-sensitive fluorescent probe capable of detecting hydrogen peroxide (H2O2) and cytosolic superoxide anions (·O2−). Since H2O2 can generate hydroxyl radicals and ·O2− can be converted to hydroxyl radicals via superoxide dismutase, this assay is suitable for measuring ROS scavenging. NAC and 5-ASA served as positive controls, validating the assay's reliability. Both NAC, a well-known antioxidant that also induces glutathione (GSH) synthesis, and 5-ASA, which strongly scavenges ROS with clinical support [20-22], confirm the appropriateness of our approach. NZ-419 significantly attenuated H2O2-induced oxidative stress; however, treatment with NZ-419 alone did not alter Nrf2 promoter activity, indicating that NZ-419 primarily influences ROS-dependent pathways.

Nrf2 is a transcription factor that plays a central role in regulating cellular antioxidant defenses. Under oxidative stress, Nrf2 dissociates from Kelch-like ECH-associated protein 1 (Keap1), which normally mediates Nrf2 ubiquitination and degradation [23]. Freed Nrf2 translocates to the nucleus, forms heterodimers, and binds antioxidant response elements to induce the expression of downstream genes, including phase II detoxifying enzymes [24].

To our knowledge, this is the first report demonstrating that a radical scavenger can inhibit intestinal polyp formation in mice. NZ-419 tended to reduce total polyp numbers in both the colon and small intestine, but a statistically significant reduction was observed only in the middle segment of the small intestine. Interestingly, while the 500 ppm NZ-419 group showed a significant decrease in polyp count, the 1000 ppm group did not; nonetheless, the polyp numbers between the two dosage groups were nearly identical, suggesting a biologically relevant effect, though the underlying cause remains unclear. Previous studies have reported that agents such as indomethacin (a COX inhibitor), nimesulide (a COX-2 selective inhibitor),

sesamol (a COX-2 suppressor), and apocynin (an NADPH oxidase inhibitor) significantly reduce polyp numbers in the middle to distal intestine [25-28]. Conversely, lipoprotein lipase activator NO-1886, peroxisome proliferator-activated receptor ligands, and erythromycin mainly suppress polyps in the proximal small intestine [29-31]. Because prior studies indicate that antioxidants and anti-inflammatory agents reduce polyp formation in the middle intestinal regions, the effects of NZ-419 are consistent with these observations, supporting the idea that its radical scavenging activity contributes to its inhibitory effect on polyp development in this region. Furthermore, analysis of oxidative stress markers (RCs) in the serum of NZ-419-treated Min mice revealed a reduction in RC levels, confirming that NZ-419 exerted antioxidative effects in vivo.

We then examined the tumor-suppressive potential of NZ-419 by assessing proliferation-related factors in both the mucosal and polyp regions of the small intestine in Min mice. Our results showed that NZ-419 treatment reduced c-Myc mRNA levels in intestinal polyps. As an oncogene, c-Myc plays a pivotal role in cell cycle progression, and its downregulation likely contributes to the observed decrease in PCNA-positive cells, indicating suppressed proliferation in neoplastic cells.

Interestingly, c-Myc is also recognized as an Nrf2 inhibitor, capable of interfering with Nrf2 signaling and the expression of downstream target genes. It can interact with the EpRE binding complex, promoting Nrf2 degradation. At this stage, it remains unclear whether NZ-419 lowers c-Myc levels directly through its radical scavenging activity or indirectly by enhancing Nrf2 expression. Further studies are necessary to elucidate the underlying mechanisms.

NZ-419 exhibits characteristics of a cancer chemopreventive agent. It is orally administrable and is currently undergoing phase II clinical trials. Additionally, NZ-419 appears to be a promising candidate for chemoprevention because it meets several key criteria: (I) convenient dosing, (II) ease of administration, (III) affordability, and (IV) minimal side effects [32].

Conclusions

In summary, our study indicates that NZ-419 inhibits intestinal polyp formation in Min mice, supporting the potential use of radical scavengers and antioxidants as cancer chemopreventive agents. Comprehensive in vitro and in vivo studies, along with clinical trials, are needed to confirm NZ-419's effectiveness in suppressing colorectal carcinogenesis. If NZ-419 is definitively proven to prevent CRC or other cancers, its impact could be substantial, particularly in the context of drug repositioning strategies.

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Conflict of interest: None.

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Ethics statement: None.

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