

Astaxanthin Inhibits *Helicobacter pylori*-induced Inflammatory and Oncogenic Responses in Gastric Mucosal Tissues of Mice

Hwana Han, Joo Weon Lim, Hyeyoung Kim

Department of Food and Nutrition, BK21 FOUR, College of Human Ecology, Yonsei University, Seoul, Korea

Helicobacter pylori is recognized as a risk factor for gastric carcinogenesis. The chronic exposure of gastric epithelium to *H. pylori* induces a prolonged inflammatory state that may progress to gastric cancer. Astaxanthin, a pinkish antioxidant carotenoid, abundant in marine organisms, is known for its protective effect against inflammation and multiple types of cancer. The purpose of this study was to examine the effect of astaxanthin on *H. pylori*-induced oxidative injury, inflammation, and oncogene expression in gastric mucosal tissues of the infected mice. Mice were inoculated using oral gavage with *H. pylori* suspension (10^8 colony forming unit of *H. pylori*/0.1 mL) for three days, after which they were fed astaxanthin-supplemented diet (5 mg/kg body weight/day for seven weeks). The effects of astaxanthin on *H. pylori*-induced increase in lipid peroxide (LPO) production, myeloperoxidase (MPO) activity, expression of the inflammatory cytokine IFN- γ and oncogenes (c-myc and cyclin D1), and the accompanying histologic changes in gastric mucosal tissues were evaluated. *H. pylori* infection increased the level of LPO, MPO activity, and the expression of IFN- γ , c-myc, and cyclin D1 in gastric mucosal tissues of mice. *H. pylori* infection induced neutrophil infiltration and hyperplasia of gastric mucosa. Astaxanthin supplementation attenuated these effects. In conclusion, consumption of astaxanthin-rich foods may prevent *H. pylori*-associated oxidative damage and inflammatory and oncogenic responses in gastric mucosal tissues.

Key Words Astaxanthin, *Helicobacter pylori*, Gastric mucosa, Inflammation, Oncogene

INTRODUCTION

Helicobacter pylori (*H. pylori*) is widely recognized as a major risk factor for gastric inflammation. Moreover, the continued gastric mucosal exposure to *H. pylori* results in a chronic inflammatory state as the host tries to protect itself from the bacterium, which in turn increases the risk of gastric carcinogenesis [1]. The levels of inflammatory cytokines, such as interleukin-8 and IFN- γ , increased in *H. pylori*-infected gastric cancer patients [2,3]. These inflammatory mediators act as chemoattractants recruiting neutrophils to the infected site and increasing the level of reactive oxygen species (ROS) in the host's cells [4]. Indeed, increased ROS production was reported in the gastric tissues of patients infected by *H. pylori* [5]. ROS are widely accepted as major pathogenic factors in *H. pylori*-induced gastric carcinogenesis, as the resulting oxidative DNA damage is considered one of the significant events heralding the progression to carcinogenesis [6]. Pre-

vious studies revealed that *H. pylori* infection increased the cellular ROS level by activating NADPH oxidase in human gastric epithelial cells [7,8]. The release of large amounts of ROS from infiltrated neutrophils by NADPH oxidase activity may contribute to the activation of redox-sensitive oncogenic signaling pathways, such as the NF- κ B and the Wnt pathway [9], which are known to regulate oncogenes at the transcriptional and post-transcriptional levels. The regulated oncogenes include c-myc and cyclin D1 [10,11], which function as key elements of cancer development in humans. Overexpression of c-myc and cyclin D1 has been observed in over 40% and 22% of gastric cancer cases, respectively [12,13]. Moreover, several in vitro studies have demonstrated that the aberrant expression of these genes is involved in the proliferation of gastric cancer cells [14,15]. Therefore, the association of *H. pylori* infection with c-myc and cyclin D1 expression in gastric mucosa needs to be clarified in order to understand *H. pylori*-mediated gastric carcinogenesis in depth.

Received November 14, 2020, Revised December 10, 2020, Accepted December 12, 2020

Correspondence to Hyeyoung Kim, E-mail: kim626@yonsei.ac.kr, <https://orcid.org/0000-0002-7019-917X>



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Astaxanthin is the ketocarotenoid responsible for the red-orange pigmentation observed in aquatic organisms such as salmon and lobsters [16]. Astaxanthin has attracted substantial interest due to its anti-inflammatory and anti-cancer effects [17]. Our previous study has revealed that astaxanthin protects gastric epithelial cells from the harmful effects of *H. pylori* infection by attenuating the induction of ROS production and increasing the activity of antioxidant enzymes [8]. These results led us to propose the hypothesis that astaxanthin may avert *H. pylori*-associated gastric alterations including oxidative damage (increase in lipid peroxide [LPO] levels and myeloperoxidase [MPO] activity) and the expression of the inflammatory cytokine IFN- γ as well as oncogenes (c-myc and cyclin D1) in gastric mucosal tissues of the infected hosts.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (6 weeks of age), purchased from Orient Bio Inc. (Seongnam, Korea), were maintained in a controlled room in the animal facility of Yonsei University College of Medicine under the following conditions: temperature, 23.0°C \pm 3.0°C; humidity, 50% \pm 10%; 12-hour light/dark cycle. Animals were housed in polypropylene cages furnished with hardwood chip bedding (5 animals per cage) and had free access to food and water. All experimental procedures were approved by the Institutional Review Board (IRB) at Animal Ethical and Experimental Committee of Yonsei University College of Medicine, Seoul, Korea (IRB No. IACUC No. 2018-0127).

Bacterial inoculation

The *H. pylori* strain NCTC 11637 (CagA+ and VacA+) was obtained from the American Type Culture Collection (Rockville, MD, USA) and stored at -80°C in BBL Brucella Broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) supplemented with 10% FBS (Gibco; Grans Island, NY, USA). Bacterial cells were grown and prepared under previously described conditions [8]. After reaching the exponential phase, bacteria were suspended in PBS at a density of 10⁹ colony forming unit (CFU)/mL. No specific pretreatments (such as acid inhibition or antibiotics) were used before orogastric *H. pylori* inoculation or before the animals were sacrificed. Mice were orogastrically inoculated 3 times (days 0, 1, and 2) with 0.1 mL of the inoculum preparation of *H. pylori* (10⁸ CFU of *H. pylori*/0.1 mL) through a feeding needle. Prior to each inoculation, mice were fasted for 12 hours. Non-inoculated control mice were administered 0.1 mL of sterile PBS under identical conditions.

Experimental protocol

The mice were randomly assigned to the following 3 groups (n = 15 per group): (1) non-infected control (None) group

comprising mice administered sterile PBS and fed standard chow (AIN-76A; Research Diets, New Brunswick, NJ, USA); (2) infected control (*H. pylori* control) group comprising mice inoculated with *H. pylori* and fed standard chow; (3) astaxanthin (*H. pylori* + ASX) group comprising mice infected with *H. pylori* and fed an AIN-76A diet supplemented with 0.005% astaxanthin (Sigma-Aldrich, St. Louis, MO, USA). The daily dose of astaxanthin was set at 5 mg/kg body weight, based on previous studies demonstrating that astaxanthin decreased the incidence of colonic adenocarcinoma in a mouse colitis model and hepatic cholesterol levels in mice fed a high-fat diet [18,19].

Mice were weighed weekly during the study period, while food intake was evaluated three times a week. Mice were killed by carbon dioxide inhalation at the end of the 7th week. Upon death, gastric mucosal tissues were collected. One half of the tissues were subjected to histological analysis. The remaining tissues were homogenized in RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 25 mM Tris pH 7.4) and used for the determination of LPO abundance, MPO activity, levels of total protein (using the Bradford assay; Bio-Rad Laboratories, Hercules, CA, USA), and protein and mRNA levels of IFN- γ , c-myc, and cyclin D1.

Assessment of LPO level and MPO activity

For evaluating the oxidative damage, the level of LPO was assessed by measuring the gastric mucosal concentration of malondialdehyde according to the method of Ohkawa et al. [20]. Results were expressed as nmol/mg protein. For the determination of the gastric mucosal accumulation of neutrophilic components, the activity of MPO, a peroxidase enzyme abundantly expressed in neutrophils, was quantified according to the modified method of Krawisz et al. [21] and expressed in U/mg protein. One unit of MPO indicates the enzymatic activity needed to reduce 1 μ mol of hydrogen peroxide per minute at 25°C.

Real-time PCR analysis

The mRNA levels of IFN- γ , c-myc, and cyclin D1 were assessed using real-time PCR. Complementary DNA (cDNA) was generated from total RNA by reverse transcription using random hexamers and MuLV reverse transcriptase (Promega; Madison, WI, USA) using the following protocol: 23°C for 10 minutes, 37°C for 60 minutes, and 95°C for 5 minutes. The cDNA was amplified with the specific primers and FAM-BHQ-labeled probes described in Table 1 under the following conditions: 45 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 20 seconds, and extension at 72°C for 30 seconds. In the first cycle, the 95°C step was extended to 3 minutes. GAPDH was used as the reference gene for data normalization.

Western blot analysis

Western blot analysis was conducted as previously described [22]. After electro-blotting, nitrocellulose membranes were probed using antibodies for c-myc (sc-40), cyclin D1 (sc-8396), IFN- γ (sc-52673), and β -actin (sc-47778; all purchased from Santa Cruz Biotechnology, Dallas, TX, USA) diluted in TBS-Tween solution containing 2% dry milk, and incubated overnight at 4°C. After washing three times with TBS-Tween, the primary antibodies were detected using horseradish peroxidase-conjugated anti-mouse secondary antibody and visualized with the enhanced chemiluminescence detection system (Santa Cruz Biotechnology). β -Actin was used as a loading control. Results were expressed as the percentage density ratio of each protein to β -actin.

Histological analysis

Half of the stomach tissues dissected from mice were fixed in freshly prepared 10% neutral-buffered formalin, embedded in paraffin, cut into 4 μ m slices, stained with hematoxylin and eosin, and subjected to morphological observation under an optical microscope. To evaluate pathological changes, microscopic images (200 \times) were examined by a single investiga-

tor who was blind to the group assignments.

Statistical analysis

All values were expressed as means \pm SEM. One-way ANOVA was carried out to assess statistical significance, followed by Newman-Keul's post-hoc test. Differences with $P < 0.05$ were considered statistically significant.

RESULTS

Astaxanthin does not change body weight gain and daily food intake in *H. pylori*-infected mice

Body weight gain in the infected control group (*H. pylori* control) tended to increase compared to the ASX group (*H. pylori* + ASX) (Fig. 1A). However, the changes in the body weight among the three groups at the end of the experiment were not significantly different. The average daily food intake did not show significant differences among the three groups (Fig. 1B).

Table 1. Mouse primers for RT-PCR

Gene	Primer	Sequence (5'-3')
GAPDH	Probe	FAM-TCATACTTCTCATGGTTCACACCCATCAG-BHQ1
	Forward	TTGCTGACAATCTTGAGGGAGTT
	Reverse	CTGCTGATGCCCCCATGT
IFN- γ	Probe	FAM-CTCACAGACCTCCAGCAT-BHQ1
	Forward	TGCCATCCATGCGGAAA
	Reverse	AGCGGGAAGAAGACTCCTCTTC
c-myc	Probe	FAM-ACCTCCGCACAGCCCCTGGT-BHQ1
	Forward	AGCGACTCTGAAGAAGAGCA
	Reverse	CGTAGTTGTGCTGGTGAGTG
Cyclin D1	Probe	FAM-TCACCATCCTTTTGCCAGTTCCTCCAG-BHQ1
	Forward	TCAAGTGGCATAGATGTGGAAGAA
	Reverse	TGGCTCTGCAGATTTTCATG

RT-PCR, real-time PCR.

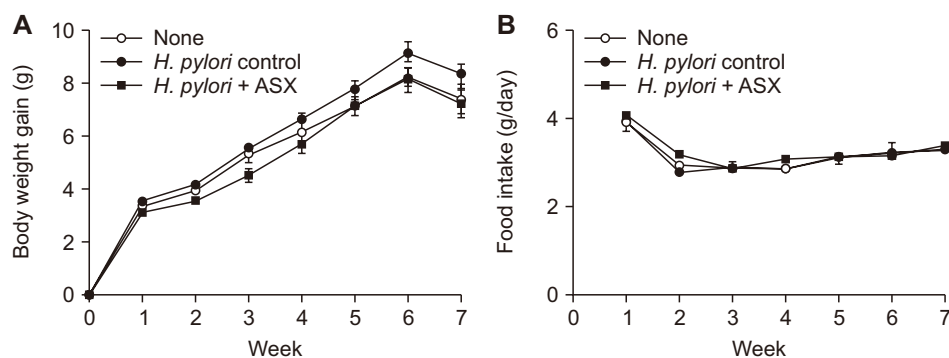


Figure 1. Effects of astaxanthin on body weight gain and daily food intake of mice infected with *H. pylori*. (A) Graph showing the weekly changes in body weight of each group. (B) Graph showing the average daily food intake of each group as measured three times a week. Values are presented as mean \pm SEM ($n = 15$ for each group). None, non-infected mice fed standard chow; *H. pylori* control, *H. pylori*-infected mice fed standard chow; *H. pylori* + ASX, *H. pylori*-infected mice fed chow supplemented with astaxanthin (ASX).

Astaxanthin decreases the level of LPO and MPO activity in *H. pylori*-infected gastric mucosa

The level of LPO reflects the oxidative stress to lipids, and, by extension, is indicative of the oxidative injury to cells. On the other hand, MPO activity is used as an index for *H. pylori*-induced accumulation of neutrophils because of the abundance of this enzyme in the infected tissues. Comparing the values of the non-infected (None) and the infected control (*H. pylori* control) groups (Fig. 2) revealed that *H. pylori* infection significantly increased both LPO level and MPO activity in gastric mucosal tissues. Notably, astaxanthin supplementation attenuated the infection-induced elevation of LPO production and MPO activity in mouse gastric mucosal tissues. These results demonstrate that astaxanthin may inhibit *H. pylori*-induced oxidative stress and neutrophil influx to mouse gastric mucosal tissues.

Astaxanthin suppresses the expression of IFN- γ , c-myc, and cyclin D1 in *H. pylori*-infected gastric mucosa

H. pylori infection significantly increased both the mRNA and protein levels of IFN- γ , whereas astaxanthin supplementation attenuated these effects (Fig. 3). The same changes, i.e., increases in the infected controls and attenuation by astaxanthin, were observed in the two examined oncogenes, c-myc and cyclin D1 (Fig. 4). These results indicate that astaxanthin can suppress *H. pylori*-mediated gastric carcinogenesis by attenuating the expression of inflammatory mediators and oncogenes in gastric mucosa.

Astaxanthin prevents *H. pylori*-induced alterations in gastric mucosa

The gastric mucosa of the uninfected (None) group displayed normal morphology, i.e., lack of inflammation or hyperplasia

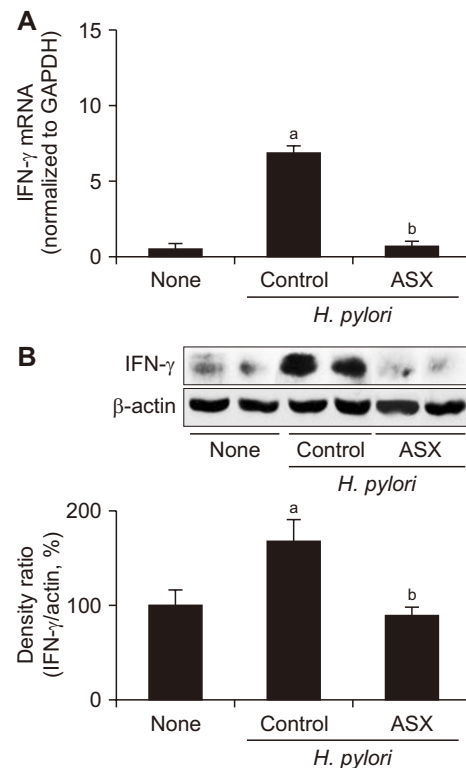


Figure 3. Effect of astaxanthin on the expression of IFN- γ in the gastric mucosal tissues of *H. pylori*-infected mice. (A) IFN- γ mRNA levels in the gastric mucosal tissues were assessed by real-time PCR. GAPDH mRNA was used to normalize the results. (B) Upper: Western blot analysis of IFN- γ . Lower: graph showing the levels of each protein as a density ratio relative to the density of the loading control (β -actin). The density ratio of the non-infected control (None) group was set as 100%. None, non-infected mice fed standard chow; Control (*H. pylori* control), *H. pylori*-infected mice fed standard chow; ASX (*H. pylori* + ASX), *H. pylori*-infected mice fed chow supplemented with astaxanthin (ASX). ^a $P < 0.05$ vs. non-infected controls (None), ^b $P < 0.05$ vs. infected controls (*H. pylori* control).

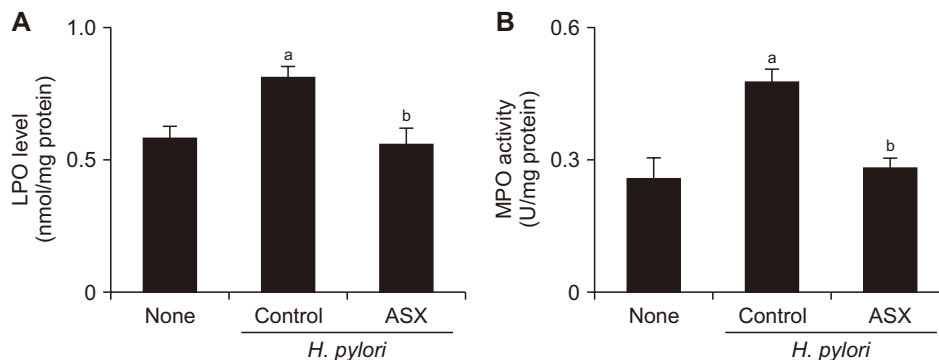


Figure 2. Effect of astaxanthin on lipid peroxide (LPO) level and myeloperoxidase (MPO) activity in the gastric mucosal tissues of *H. pylori*-infected mice. (A) Graph showing the level of LPO in each of the three groups, expressed as nmol/mg of protein. (B) Graph showing MPO activity in each of the three groups, expressed as units/mg protein. Values are presented as mean \pm SEM ($n = 5$ for each group). None, non-infected mice fed standard chow; Control (*H. pylori* control), *H. pylori*-infected mice fed standard chow; ASX (*H. pylori* + ASX), *H. pylori*-infected mice fed chow supplemented with astaxanthin (ASX). ^a $P < 0.05$ vs. None, ^b $P < 0.05$ vs. infected controls (*H. pylori* control).

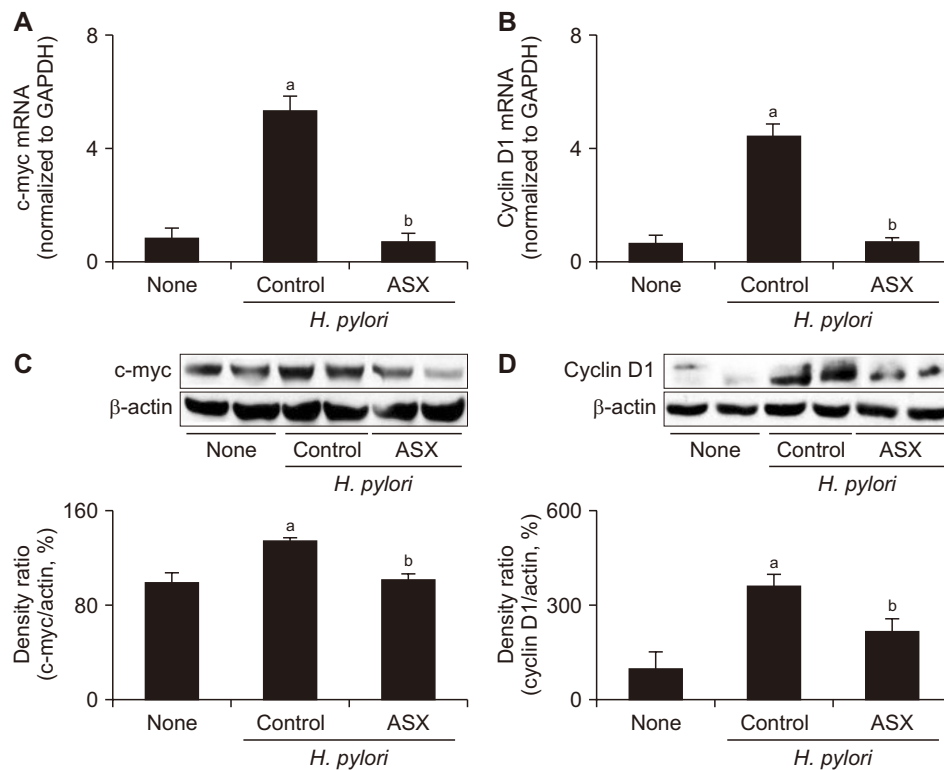


Figure 4. Effect of astaxanthin on the expression of c-myc and cyclin D1 in the gastric mucosal tissues of *H. pylori*-infected mice. (A, B) c-myc and cyclin D1 mRNA levels in the gastric mucosal tissues as assessed by RT-PCR; GAPDH mRNA was used to normalize the results. (C, D) Upper: Western blot analysis of c-myc and cyclin D1. Lower: graph showing the levels of each protein as a density ratio relative to the density of the loading control (β -actin). The density ratio of the None group was set as 100%. None, non-infected mice fed standard chow; Control (*H. pylori* control), *H. pylori*-infected mice fed standard chow; ASX (*H. pylori* + ASX), *H. pylori*-infected mice fed chow supplemented with astaxanthin (ASX). ^a $P < 0.05$ vs. non-infected controls (None), ^b $P < 0.05$ vs. infected controls (*H. pylori* control).

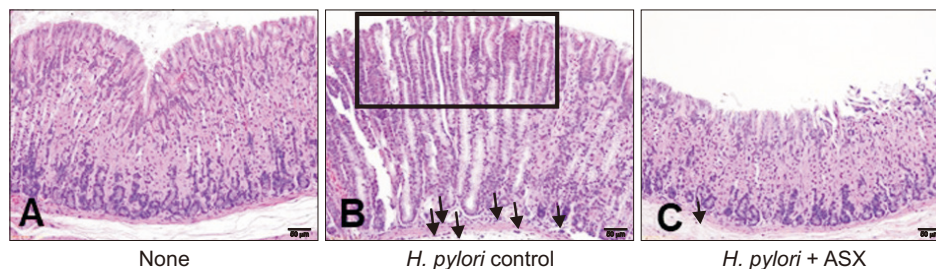


Figure 5. Effect of astaxanthin on *H. pylori*-induced gastric mucosal damage. (A) Normal gastric tissue from None group. (B) Abnormal gastric tissue from *H. pylori* control groups. Focal hyperplastic glands (square) and infiltration of neutrophils (arrows) are observed. (C) Gastric mucosal tissues from *H. pylori* + ASX group. Both the presence of focal hyperplastic glands and the infiltration by neutrophils are less profound than in (B). All images are hematoxylin and eosin-stained gastric mucosal sections viewed under an optical microscope at 200 \times . The bar represents a length of 50 μ m. None, non-infected mice fed standard chow; *H. pylori* control, *H. pylori*-infected mice fed standard chow; *H. pylori* + ASX, *H. pylori*-infected mice fed chow supplemented with astaxanthin (ASX).

(Fig. 5A). In contrast, the infected control (*H. pylori* control) group had mild inflammation and hyperplasia, as indicated by the presence of focal hyperplastic glands and the infiltration of neutrophils in the gastric mucosa (Fig. 5B). Infected mice fed an astaxanthin-supplemented diet (*H. pylori* + ASX) exhibited reduced neutrophil infiltration and hyperplasia compared to those of the infected control group (Fig. 5C). These results

suggest that astaxanthin supplementation might exert a preventive effect against *H. pylori*-induced gastric alterations.

DISCUSSION

Oxidative stress in the gastric mucosa resulting from *H. pylori* infection has a crucial role in gastric carcinogenesis. *H. pylori*

infection is highly correlated with increased oxidative damage to the gastric mucosa [1]. In particular, the elevated production of ROS enhances the generation of LPO, as they react with polyunsaturated fatty acids in the cellular membrane and promote the oxidative degradation of the lipids [23]. Therefore, the increased LPO level observed in this study after the inoculation with *H. pylori* may be attributed to infection-induced oxidative damage to gastric mucosa cells. Since astaxanthin treatment reversed this effect, we conclude that its antioxidative activity might protect gastric tissues from the oxidative damage induced by *H. pylori* infection. Our results concur with those of previous studies in which the oral administration of astaxanthin was shown to prevent the elevation of the LPO level in the gastric tissues in a mouse model of gastric ulceration [24,25].

H. pylori-induced inflammation is characterized by the persistent infiltration of neutrophils in gastric mucosa, which contributes to the pathologies of gastric disorders, particularly by releasing inflammatory mediators and ROS [26]. This was confirmed in our study, as MPO activity, an indicator of neutrophil influx in tissues, was elevated by *H. pylori* infection. Importantly, this increase was mitigated by astaxanthin treatment.

Increased expression of IFN- γ after *H. pylori* infection has been observed in the stomach tissues of Mongolian gerbils [27] and humans [3]. *H. pylori* is known to stimulate the production of IFN- γ from peripheral blood mononuclear cells and lymphocytes in gastric tissue [28]. A role for IFN- γ in *H. pylori*-induced gastric inflammation is supported by the finding that infected IFN- γ ^{-/-} mice develop less severe gastritis compared with their wild-type counterparts [29]. In the current study, *H. pylori* infection increased the mRNA and protein levels of IFN- γ in mouse gastric mucosa, whereas astaxanthin intake attenuated this increase. These findings are consistent with the previous observation that the dietary intake of astaxanthin can reduce the release of IFN- γ in splenocytes extracted from *H. pylori*-infected mice [30], and suggest that astaxanthin supplementation might alleviate the gastric inflammation by reducing *H. pylori*-induced IFN- γ expression.

Several studies have shown that *H. pylori* infection increases the expression of c-myc and cyclin D1 and induces hyperproliferation in gastric epithelial AGS cells [14,31]. Since the transcription factor c-myc suppresses the cell cycle arrest and enhances cell proliferation, the deregulation of its expression is a pivotal event in the pathological process leading to cancer [32]. On the other hand, cyclin D1 plays a major role in cell-cycle control as it allows the cycle to progress from the G1 to the S phase. Abnormal cyclin D1 expression is known to participate in cancer development and progression by increasing the rate of cellular proliferation [33]. Our data show that astaxanthin supplementation attenuates the increases in the mRNA and protein levels of c-myc and cyclin D1 in mouse gastric tissues that would otherwise result from *H. pylori* infection. These results are in line with previous findings

illustrating that astaxanthin decreases the expression of both proteins and inhibits the proliferation of human gastric cancer and colon cancer cells [34,35].

We have previously demonstrated that activation of NF- κ B and AP-1 mediates hyperproliferation by inducing β -catenin and c-myc in *H. pylori*-infected gastric epithelial cells [14]. Therefore, NF- κ B and β -catenin signaling may mediate hyper-proliferation via induction of c-myc and cyclins. *H. pylori* infection increased expression of c-myc and cyclin D1 through activation of NF- κ B [14,36]. We also showed that astaxanthin inhibited *H. pylori*-induced NF- κ B activation in gastric epithelial cells [8]. The antioxidant β -carotene inhibited *H. pylori*-induced cell proliferation and the expression of c-myc and cyclin E by reducing the levels of β -catenin and phosphorylated glycogen synthase kinase 3 β [37]. Lycopene inhibited oxidative stress-mediated activation of Wnt/ β -catenin signaling including alteration of Wnt/ β -catenin multiprotein complex molecules and attenuated hyper-proliferation in gastric epithelial cells [38]. These studies suggest that astaxanthin supplement may suppress *H. pylori*-induced expression of c-myc and cyclin D1 through inhibition of NF- κ B activation and Wnt/ β -catenin signaling in gastric mucosal tissues.

Jin et al. [39] and Luo et al. [40] demonstrated that 6- to 7-week-treatment of *H. pylori* induced erosions and infiltration of inflammatory cells, and hyperplasia in gastric mucosal tissues of mice. Our results showed that 7-week-treatment of *H. pylori* caused inflammation and hyperplasia of gastric tissues of the infected mice.

In conclusion, astaxanthin may suppress oxidative gastric tissue damage and the expression of the inflammatory cytokine, IFN- γ , and the oncogenes, c-myc and cyclin D1, in *H. pylori*-infected gastric mucosal tissues. These results suggest that astaxanthin supplementation might have a preventive effect against *H. pylori*-associated gastric carcinogenesis.

ACKNOWLEDGMENTS

This study was supported financially by a grant from the National Research Foundation (NRF) of Korea, which is funded by the Korean Government (NRF-2018R1A2B2005575). Authors are grateful to Professor Joo Young Kim (Dept. Of Pharmacology, Yonsei University College of Medicine, Seoul, Republic of Korea) for her kind advice and technical assistance in animal experimentation.

CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ORCID

Hwana Han, <https://orcid.org/0000-0003-0705-125X>
 Joo Weon Lim, <https://orcid.org/0000-0001-7483-3820>
 Hyeyoung Kim, <https://orcid.org/0000-0002-7019-917X>

REFERENCES

- Butcher LD, den Hartog G, Ernst PB, Crowe SE. Oxidative stress resulting from *Helicobacter pylori* infection contributes to gastric carcinogenesis. *Cell Mol Gastroenterol Hepatol* 2017;3:316-22.
- Bartchewsky W Jr, Martini MR, Masiero M, Squassoni AC, Alvarez MC, Ladeira MS, et al. Effect of *Helicobacter pylori* infection on IL-8, IL-1beta and COX-2 expression in patients with chronic gastritis and gastric cancer. *Scand J Gastroenterol* 2009;44:153-61.
- Abdollahi H, Shams S, Zahedi MJ, Darvish Moghadam S, Hayatbakhsh MM, Jafarzadeh A. IL-10, TNF- α and IFN- γ levels in serum and stomach mucosa of *Helicobacter pylori*-infected patients. *Iran J Allergy Asthma Immunol* 2011;10:267-71.
- Crabtree JE, Farmery SM. *Helicobacter pylori* and gastric mucosal cytokines: evidence that CagA-positive strains are more virulent. *Lab Invest* 1995;73:742-5.
- Davies GR, Simmonds NJ, Stevens TR, Sheaff MT, Banatvala N, Laurenson IF, et al. *Helicobacter pylori* stimulates antral mucosal reactive oxygen metabolite production in vivo. *Gut* 1994;35:179-85.
- Farinati F, Cardin R, Degan P, Rugge M, Mario FD, Bonvicini P, et al. Oxidative DNA damage accumulation in gastric carcinogenesis. *Gut* 1998;42:351-6.
- Byun E, Lim JW, Kim JM, Kim H. α -Lipoic acid inhibits *Helicobacter pylori*-induced oncogene expression and hyperproliferation by suppressing the activation of NADPH oxidase in gastric epithelial cells. *Mediators Inflamm* 2014;2014:380830.
- Kim SH, Lim JW, Kim H. Astaxanthin inhibits mitochondrial dysfunction and interleukin-8 expression in *Helicobacter pylori*-infected gastric epithelial cells. *Nutrients* 2018;10:1320.
- Korswagen HC. Regulation of the Wnt/beta-catenin pathway by redox signaling. *Dev Cell* 2006;10:687-8.
- Hinz M, Krappmann D, Eichten A, Heder A, Scheidereit C, Strauss M. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol* 1999;19:2690-8.
- Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999;398:422-6.
- Calcagno DQ, Leal MF, Assumpcao PP, Smith MA, Burbano RR. MYC and gastric adenocarcinoma carcinogenesis. *World J Gastroenterol* 2008;14:5962-8.
- Blok P, Craanen ME, van Diest PJ, Dekker W, Tytgat GN. Lack of cyclin D1 overexpression in gastric carcinogenesis. *Histopathology* 2000;36:151-5.
- Byun E, Park B, Lim JW, Kim H. Activation of NF- κ B and AP-1 mediates hyperproliferation by inducing β -catenin and c-myc in *Helicobacter pylori*-infected gastric epithelial cells. *Yonsei Med J* 2016;57:647-51.
- Luo J, Yan R, He X, He J. Constitutive activation of STAT3 and cyclin D1 overexpression contribute to proliferation, migration and invasion in gastric cancer cells. *Am J Transl Res* 2017;9:5671-7.
- Davinelli S, Nielsen ME, Scapagnini G. Astaxanthin in skin health, repair, and disease: a comprehensive review. *Nutrients* 2018;10:522.
- Yuan JP, Peng J, Yin K, Wang JH. Potential health-promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. *Mol Nutr Food Res* 2011;55:150-65.
- Nakao R, Nelson OL, Park JS, Mathison BD, Thompson PA, Chew BP. Effect of dietary astaxanthin at different stages of mammary tumor initiation in BALB/c mice. *Anticancer Res* 2010;30:2171-5.
- Wang J, Liu S, Wang H, Xiao S, Li C, Li Y, et al. *Xanthophyllomyces dendrorhous*-derived astaxanthin regulates lipid metabolism and gut microbiota in obese mice induced by a high-fat diet. *Mar Drugs* 2019;17:337.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Assessment of inflammation in rat and hamster models. *Gastroenterology* 1984;87:1344-50.
- Kim A, Lim JW, Kim H, Kim H. Supplementation with *Angelica keiskei* inhibits expression of inflammatory mediators in the gastric mucosa of *Helicobacter pylori*-infected mice. *Nutr Res* 2016;36:488-97.
- Kwiecien S, Jasnos K, Magierowski M, Sliwowski Z, Pajdo R, Brzozowski B, et al. Lipid peroxidation, reactive oxygen species and antioxidative factors in the pathogenesis of gastric mucosal lesions and mechanism of protection against oxidative stress - induced gastric injury. *J Physiol Pharmacol* 2014;65:613-22.
- Kim JH, Kim YS, Song GG, Park JJ, Chang HI. Protective effect of astaxanthin on naproxen-induced gastric antral ulceration in rats. *Eur J Pharmacol* 2005;514:53-9.
- Wang X, Willén R, Wadström T. Astaxanthin-rich algal meal and vitamin C inhibit *Helicobacter pylori* infection in BALB/cA mice. *Antimicrob Agents Chemother* 2000;44:2452-7.
- Robinson K, Argent RH, Atherton JC. The inflammatory and immune response to *Helicobacter pylori* infection. *Best Pract Res Clin Gastroenterol* 2007;21:237-59.
- Crabtree JE, Court M, Aboshkiwa MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to *Helicobacter pylori* infection in Mongolian gerbils. *J Pathol* 2004;202:197-207.
- Fan XJ, Chua A, Shahi CN, McDevitt J, Keeling PW, Kelleher D. Gastric T lymphocyte responses to *Helicobacter pylori* in patients with H pylori colonisation. *Gut* 1994;35:1379-84.
- Smythies LE, Waites KB, Lindsey JR, Harris PR, Ghiara P, Smith PD. *Helicobacter pylori*-induced mucosal inflammation is Th1 mediated and exacerbated in IL-4, but not IFN-gamma, gene-deficient mice. *J Immunol* 2000;165:1022-9.
- Bennedson M, Wang X, Willén R, Wadström T, Andersen LP. Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. *Immunol Lett* 1999;70:185-9.
- Hirata Y, Maeda S, Mitsuno Y, Akanuma M, Yamaji Y, Ogura K,

- et al. Helicobacter pylori activates the cyclin D1 gene through mitogen-activated protein kinase pathway in gastric cancer cells. Infect Immun 2001;69:3965-71.
32. Vita M, Henriksson M. The Myc oncoprotein as a therapeutic target for human cancer. Semin Cancer Biol 2006;16:318-30.
 33. John RR, Malathi N, Ravindran C, Anandan S. Mini review: multifaceted role played by cyclin D1 in tumor behavior. Indian J Dent Res 2017;28:187-92.
 34. Palozza P, Torelli C, Boninsegna A, Simone R, Catalano A, Mele MC, et al. Growth-inhibitory effects of the astaxanthin-rich alga Haematococcus pluvialis in human colon cancer cells. Cancer Lett 2009;283:108-17.
 35. Kim JH, Park JJ, Lee BJ, Joo MK, Chun HJ, Lee SW, et al. Astaxanthin inhibits proliferation of human gastric cancer cell lines by interrupting cell cycle progression. Gut Liver 2016;10:369-74.
 36. Yang GF, Deng CS, Xiong YY, Gong LL, Wang BC, Luo J. Expression of nuclear factor-kappa B and target genes in gastric precancerous lesions and adenocarcinoma: association with Helicobacter pylori cagA (+) infection. World J Gastroenterol 2004;10:491-6.
 37. Kim D, Lim JW, Kim H. β -carotene inhibits expression of c-Myc and Cyclin E in *Helicobacter pylori*-infected gastric epithelial cells. J Cancer Prev 2019;24:192-6.
 38. Park B, Lim JW, Kim H. Lycopene treatment inhibits activation of Jak1/Stat3 and Wnt/ β -catenin signaling and attenuates hyperproliferation in gastric epithelial cells. Nutr Res 2019;70:70-81.
 39. Jin DZ, Jung HC, Kim JM, Kim JS, Song IS, Kim CY. Establishment of BALB/c mice model infected with *Helicobacter pylori*. Korean J Intern Med 1999;14:55-63.
 40. Luo J, Song J, Zhang H, Zhang F, Liu H, Li L, et al. Melatonin mediated Foxp3-downregulation decreases cytokines production via the TLR2 and TLR4 pathways in *H. pylori* infected mice. Int Immunopharmacol 2018;64:116-22.