World Journal of Gastrointestinal Pathophysiology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4291/wjgp.v7.i4.300

World J Gastrointest Pathophysiol 2016 November 15; 7(4): 300-306
ISSN 2150-5330 (online)

ORIGINAL ARTICLE

Basic Study

Anti-*Helicobacter pylori* effect of CaG-NANA, a new sialic acid derivative

Yun-Hee Rhee, Hyun-Jeong Ku, Hye-Ji Noh, Hyang-Hyun Cho, Hee-Kyong Kim, Jin-Chul Ahn

Yun-Hee Rhee, Hyun-Jeong Ku, Jin-Chul Ahn, Beckman Laser Institute Korea, Dankook University, Chungnam 31116, South Korea

Hye-Ji Noh, Hyang-Hyun Cho, Hee-Kyong Kim, MEDINUTROL Co., Jeonnam 57024, South Korea

Jin-Chul Ahn, Department of Biomedical Science, College of Medicine, Dankook University, Chungnam 31116, South Korea

Jin-Chul Ahn, Medical Laser Research Center, Dankook University, Chungnam 31116, South Korea

Author contributions: Rhee YH performed the majority of experiments, analyzed the data, and wrote the manuscript; Ku HJ and Noh HJ participated in animal experiments; Cho HH, Kim HK and Ahn JC designed and coordinated the research.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Dankook University (IACUC protocol number: DKU-14-036).

Animal care and use statement: The ICR mice (20-22 g) were housed in pathogen free environment and had free access to sterile neutral water and standard mouse feed. Oral administration was performed with conscious animals using sonde appropriate for the animal size.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Data sharing statement: Statistics and histology analysis of data are available from the corresponding author at jcahn@dankook.ac.kr.

Manuscript source: Invited manuscript

Correspondence to: Jin-Chul Ahn, MD, Department of Biomedical Science, College of Medicine, Dankook University, 119 Dandae-ro, Cheonan, Chungnam 31116, South Korea. jcahn@dankook.ac.kr
Telephone: +82-1899-3700

Received: June 28, 2016
Peer-review started: June 29, 2016
First decision: August 10, 2016
Revised: August 12, 2016
Accepted: September 13, 2016
Article in press: September 18, 2016
Published online: November 15, 2016

Abstract

AIM

To investigate the bactericidal effects of calcium chelated N-acetylneuraminic acid-glycomacropeptide (CaG-NANA) against *Helicobacter pylori* (*H. pylori*).

METHODS

For manufacture of CaG-NANA, calcium (Ca) was combined with glycomacropeptide (GMP) by chelating, and N-acetylneuraminic acid (NANA) was produced with Ca-GMP substrate by an enzymatic method. The final concentration of each component was 5% Ca, 7% NANA, 85% GMP, and 3% water. For *in vitro* study, various concentrations of CaG-NANA were investigated under the minimal inhibitory concentration (MIC). For *in vivo* study, CaG-NANA was administered orally for 3 wk after *H. pylori* infection. The levels of inflammatory cytokines in blood were analyzed by enzyme-linked immunosorbent assay and eradication of *H. pylori* was assessed by histological observation.

RESULTS

The time-kill curves showed a persistent decrease in cell

numbers, which depended on the dose of CaG-NANA, and MIC of CaG-NANA against *H. pylori* was 0.5% *in vitro*. Histopathologic observation revealed no obvious inflammation or pathologic changes in the gastric mucosa in the CaG-NANA treatment group *in vivo*. The colonization of *H. pylori* was reduced after CaG-NANA treatment. The levels of interleukin (IL)-6, IL-1β, tumor necrosis factor-α, and IL-10 were also decreased by CaG-NANA.

CONCLUSION

CaG-NANA demonstrates effective anti-bactericidal activity against *H. pylori* both *in vitro* and *in vivo*.

Key words: *Helicobacter pylori*; Calcium chealated N-acetylneuraminic acid-glycomacropeptide

Core tip: Calcium chelated N-acetylneuraminic acid-glycomacropeptide demonstrates effective anti-bactericidal activity against *Helicobacter pylori* both *in vitro* and *in vivo*.

Rhee YH, Ku HJ, Noh HJ, Cho HH, Kim HK, Ahn JC. Anti-*Helicobacter pylori* effect of CaG-NANA, a new sialic acid derivative. *World J Gastrointest Pathophysiol* 2016; 7(4): 300-306 Available from: URL: http://www.wjgnet.com/2150-5330/full/v7/i4/300.htm DOI: http://dx.doi.org/10.4291/wjgp.v7.i4.300

INTRODUCTION

Helicobacter pylori (*H. pylori*) has been reported to be associated with many gastrointestinal diseases such as chronic gastritis, peptic ulcers, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma[1-3]. Until now, standard triple therapy consisting of a proton pump inhibitor and two broad-spectrum antibiotics, usually amoxicillin, clarithromycin or metronidazole, has been able to achieve eradication[3-5]. However, the Maastricht IV Consensus report recommended the careful choice of the antibiotic combination for treatment according to local *H. pylori* antibiotic resistance patterns due to multidrug resistance of *H. pylori*. For example, the concentrations of antibiotics for treatment of *H. pylori* infection were required at four times when *H. pylori* had local clarithromycin resistance[6]. Since antibiotic abuse had side effects and allowed development of resistance to antibiotics, alternative strategies have been proposed to counteract *H. pylori* infection. As an alternative approach, preservation of mucus from *H. pylori* attachment is emphasized because *H. pylori* infection disrupted the epithelial barrier which resulted in inflammation or cancer[7]. For example, dietary inhibitors such as lacto-oligosaccharide has been suggested as a solution for *H. pylori* infections[8]. Although oligosaccharides specific for the *H. pylori* lectins may potentially act as inhibitors of adhesion to mucus, their production in commercial amounts as anti-adhesion therapeutic agents is still a problem[9,10]. Here, we introduce a new N-acetylneuraminic acid (NANA) combined glycomacropeptide (GMP) which was made from milk serum hydrolysis protein powder as an anti-*H. pylori* agent. Wadström *et al*[11] reported that NANA derivative had an adhesion potential to *H. pylori* lectins and Hirmo *et al*[12] reported that milk glycoprotein had an anti-*H. pylori* effect. We designed a new material with NANA, GMP, and calcium (CaG-NANA), and anti-*H. pylori* activities of CaG-NANA were investigated both *in vitro* and *in vivo*.

MATERIALS AND METHODS

H. pylori strain and experimental animals

H. pylori strain (SS1-passed-5) was kindly provided by Helicobacter pylori Korean Type Culture Collection (HpKTCC, Jinju, South Korea) and grown in 1.5% agar added Brucella broth with 10% horse serum (No. CM0169; OXOID, Waltham, MA, United States). The pathogen-free (SPF) male ICR mice (20-22 g) were purchased from Orient Bio (Daejeon, South Korea). All the animals were housed in an SPF environment and had free access to sterile neutral water and standard mouse feed. The experimental procedures in this study were approved by the Experimental Animal Ethics Committee of Dankook University, South Korea (No. DKU14-036).

Supply of CaG-NANA

CaG-NANA and each component of CaG-NANA were provided by MediNutrol (Kwangju, South Korea). As shown in Figure 1A, CaG-NANA was manufactured from calcium (Ca), GMP, and NANA by chelating and enzyme methods where the component concentrations were 5%, 85%, and 7%, respectively. For comparing each produced compound, we wrote abbreviated form as standard NANA (S-NANA), GMP linked NANA (G-NANA), and calcium chelated G-NANA (CaG-NANA).

High performance liquid chromatography for confirmation of NANA

For confirmation of NANA component, we analyzed CaG-NANA using high performance liquid chromatography (HPLC) method. HPLC analysis was performed using Agilent 1260 model equipped with a pump (G1311C), an auto sampler (G1329B), a column (G1316A), and a ultraviolet detector (G1314F), which was purchased from Agilent (Santa Clara, CA, United States). The condition of analysis was described in Table 1.

Culture and collection of *H. pylori*

H. pylori was incubated in Brucella medium contained the selective supplements (No. SR0083; OXOID, Waltham, MA, United States) under microaerobic environment (15% CO_2, 5% O_2, 80% N_2) at 37 ℃ for 72 h. The bacterial colonies were collected and identified

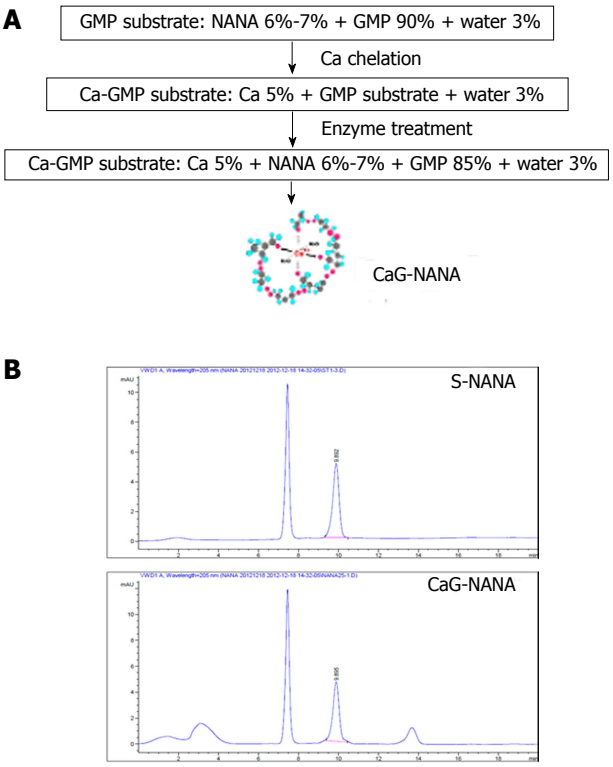

Figure 1 Confirmation of N-acetylneuraminic acid. A: The scheme of CaG-NANA manufacture; B: HPLC analysis of NANA content in CaG-NANA. The condition of HPLC analysis is described in Table 1. GMP: Glycomacropeptide; HPLC: High performance liquid chromatography; CaG-NANA: Calcium-glycomacropeptide-N-acetylneuraminic acid; S-NANA: Standard N-acetylneuraminic acid.

with a Pronto-Dry infection kit (Kokab Enterprise, Karachi, Pakistan) for bactericidal tests *in vitro*.

Inhibitory effect of CaG-NANA on H. pylori in vitro

The inhibitory activity of CaG-NANA against the growth of *H. pylori* was assessed using an agar dilution method. Briefly, 0.1%, 0.25%, or 0.5% of CaG-NANA and its components were added to the Brucella agar. The non-drug agar served as a negative control. Agar plates were inoculated with *H. pylori* at serial concentrations of 1×10^8, 1×10^7, and 1×10^6 colony forming units (CFU)/mL and cultured for 72 h. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of CaG-NANA required for complete inhibition of *H. pylori* growth. The colonies were counted using image J (https://imagej.nih.gov/ij/) after 72 h incubation and the average number was calculated. Bactericidal activity was evaluated using time-kill curves with 0.5, 1.0 and 2.0 × MIC of CaG-NANA compared with the blank controls.

Experimental design in vivo

Mice (n = 40) were randomly divided into four groups: Blank control, *H. pylori* infected control, antibiotic treatment, and CaG-NANA treatment. *H. pylori* (1×10^9 /mL) was administered by gastric intubation to mice three times in a week except the blank control, and *H. pylori* infection was detected with gastric irrigation from randomly selected mice using an *H. pylori* detection test kit which was purchased from Pronto Dry (Brignais, France; Supple 1A). The antibiotic treatment was performed with a suspension of amoxicillin (12.33 mg/kg), metronidazole (164.40 mg/kg), and clarithromycin (205.54 mg/kg) which were equivalent to clinical administration (Dankook University hospital, Korea). Treatments with CaG-NANA and each component including NANA, S-NANA, and G-NANA were performed by oral administration every day for 3 wk, and the experimental design is shown in Figure 2A.

Table 1 High performance liquid chromatography analysis condition

Detector	UV detector
Wavelength	205 nm
Column	Aminex HPX-87H Ion Exclusion column (300 mm × 7.8 mm, 9 μm)
Mobile phase	10 mmol/L H_2SO_4 in water (isocratic)
Running time	20 min
Flow rate	0.5 mL/min
Injection volume	10 mL
Temperature	40 ℃

Histological observation

The animals were deprived of feed but allowed free access to water for 24 h before sacrificed. At the end of sacrifice, blood was collected from mouse and gastric tissues were removed and fixed in 10% formalin. After fixation, tissues were processed and embedded in paraffin. The paraffin blocks were cut into 4 μm sections and stained with Harris' hematoxylin and eosin for histological observation under a microscope (BX51, Olympus, Miami, FL, United States).

Enzyme-linked immunosorbent assay

The expression levels of interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, and IL-10 in blood serum were determined using enzyme-linked immunosorbent assay (ELISA). Mouse ELISA kits were purchased from R&D Systems (Minneapolis, MN, United States). The assays were performed according to the manufacturer's instructions and repeated in triplicate.

Statistical analysis

Eradication rates were compared among groups by one-way analysis of variance (Kruskal-Wallis) and Dunn's multiple comparison test (GraphPad Software Inc., La Jolla, CA, United States). $P < 0.05$ was considered statistically significant.

RESULTS

Confirmation of NANA

As shown Figure 1B, the NANA component of CaG-NANA showed the same purity as standard NANA.

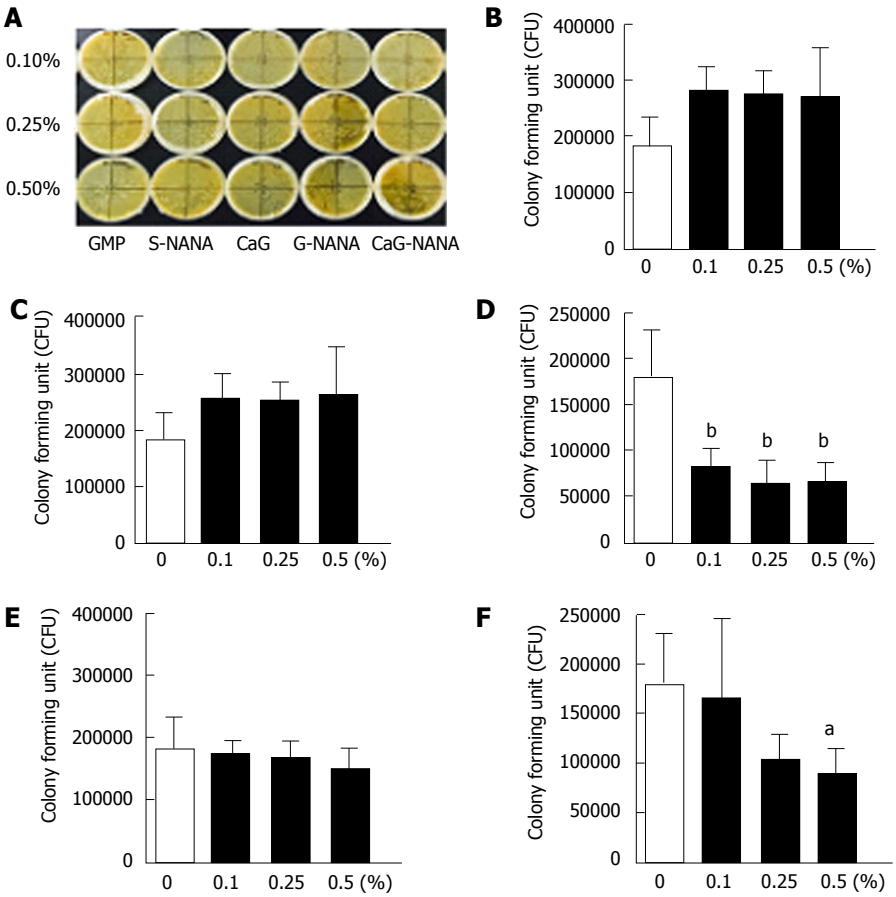

Figure 2 Inhibitory effect of calcium-glycomacropeptide-N-acetylneuraminic acid on *Helicobacter pylori in vitro*. Agar plates were inoculated with *Helicobacter pylori* (*H. pylori*) at serial concentrations of 1 × 10^8, 1 × 10^7, and 1 × 10^6 CFU/mL and cultured for 72 h. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of materials required for complete inhibition of *H. pylori* growth. Bactericidal activity was evaluated using time-kill curves with 0.5, 1.0 and 2.0 × MIC of CaG-NANA compared with blank controls. All experiments were performed three times and significance was set at $^aP < 0.1$ and $^bP < 0.05$. A: Picture of colony forming unit assay; B: GMP; C: CaG; D: S-NANA; E: G-NANA; F: CaG-NANA. GMP: Glycomacropeptide; CaG: Calcium-glycomacropeptide; S-NANA: Ststandard N-acetylneuraminic acid; CaG-NANA: Calcium-glycomacropeptide-N-acetylneuraminic acid.

Inhibitory effect of CaG-NANA on H. pylori in vitro

The bactericidal effects of CaG-NANA and each component against *H. pylori* were assessed *in vitro*. We described previously that CaG-NANA is composed of NANA, GMP and calcium, thus we tested the anti-bacterial effects of every component used in CaG-NANA synthesis. As shown in Figure 1, S-NANA, G-NANA and CaG-NANA had an anti-bacterial effect (Figure 1D, E and F) whereas GMP and GMP with calcium (CaG) had no activity in the colony forming assay (Figure 1B and C). This result indicated that only NANA included compound had anti-bacterial activity.

Inhibitory effect of CaG-NANA on H. pylori in vivo

For the *in vivo* study, we divided mice into four groups and 1 × 10^9/mL CFU of *H. pylori* was administered into mice three times for one week except the negative group. *H. pylori* infection was confirmed by gastric irrigation using an *H. pylori* detection test kit (Pronto Dry). After confirmation, treatments with antibiotics, CaG-NANA and its components were orally administered every day for 3 wk. The doses of antibiotics were described in the "Material and Methods" section (Figure 3A). The doses of CaG-NANA and its components were fixed at 0.5% (v/w). We followed the mouse weight after treatment. As shown in Figure 3B, mouse weight was decreased in the S-NANA and *H. pylori* positive control groups. From this result, S-NANA was considered to have toxicity at 0.5% of concentration.

Next, we observed the gastric mucus layer of mice. The majority of the *H. pylori* population reside in the mucus which binds the organisms *via* specific interactions such as pathogen adherence. Normal gastric mucosa has a uniform surface epithelium (Figure 4A), whereas *H. pylori* infected gastric mucosa has an irregular outline of the epithelium layer along with neutrophil and macrophage infiltrations (Figure 4B). In addition, bacterial attachment is mediated by outer membrane adhesions that bind to glycoconjugates present in the gastric mucus layer, lining the surface epithelium of the gastric mucosa in the *H. pylori* infected group. As shown in Figure 4C, the antibiotic treatment group had decreased infiltration of macrophages and adhesion of *H. pylori* colonization, however, the destruction of surface epithelium layers was also observed. Meanwhile, every NANA including group showed uniformed surface epithelium layers. Interestingly, many macrophages and neutrophils were observed in the S-NANA treatment

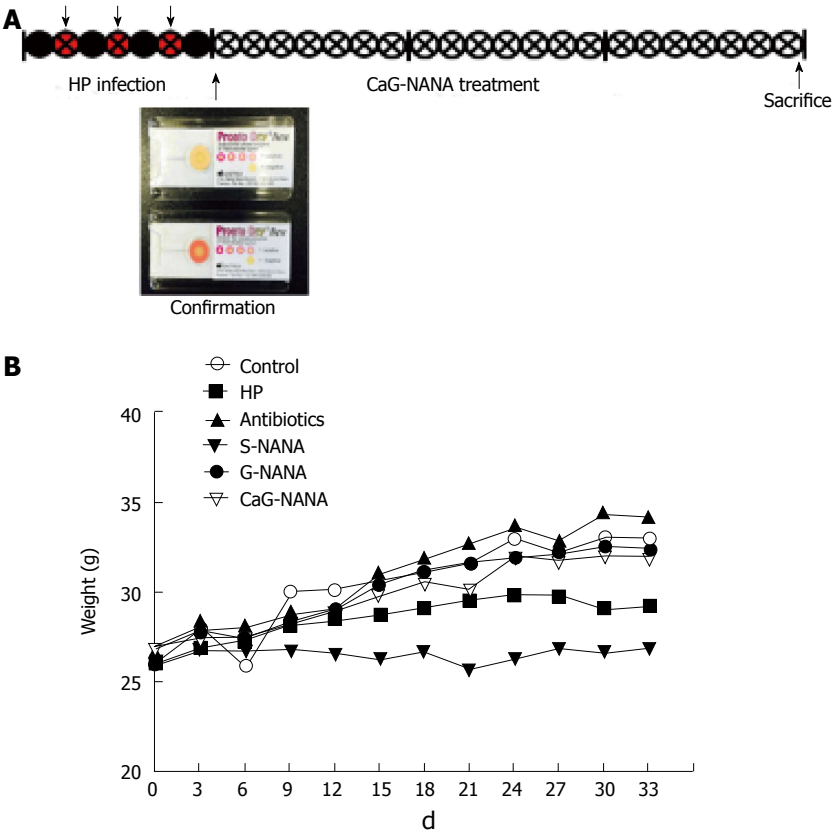

Figure 3 Inhibitory effect of calcium-glycomacropeptide-N-acetylneuraminic acid on *Helicobacter pylori in vivo*. Antibiotic doses are described in the "materials and methods" section. A: Schematic design of *in vivo* study; B: Weight changes of mice. HP: Helicobacter pyroli; S-NANA: Standard N-acetyln-euraminic acid; G-NANA: Glycomacropeptide-N-acetylneuraminic acid; CaG-NANA: Calcium-glycomacropeptide-N-acetylneuraminic acid.

group (Figure 4D). The damage to surface epithelium in the antibiotics treatment group and inflammation observed in the S-NANA treatment group might be related to mouse weight loses. Both the G-NANA and CaG-NANA treatment groups showed the detachment of *H. pylori* colonization without any damage to surface epithelium or inflammation (Figure 4E and F).

We also assessed the levels of IL-1β, IL-6, TNF-α, and IL-10 in blood by ELISA. PBS was used as a control because every compound was dissolved in PBS. Both of S-NANA and CaG-NANA showed a potent ability to decrease inflammatory cytokines. The level of IL-1β was reduced from 7.6 ng/mL to 0.8 ng/mL after S-NANA treatment and 2.36 ng/mL after CaG-NANA treatment (Figure 5A). The level of IL-6 was remarkedly decreased from 113.4 ng/mL to 33.5 ng/mL after S-NANA treatment and 1.26 ng/mL after CaG-NANA treatment (Figure 5B). The level of TNF-α was also reduced to 8.21 ng/mL after S-NANA treatment and 1.44 ng/mL after CaG-NANA treatment (Figure 5C). Meanwhile, the level of IL-10 was reduced only in the CaG-NANA treatment group from 7.62 ng/mL to 6.3 ng/mL (Figure 5D).

DISCUSSION

The anti-*H. pylori* effects of antibiotic formulas have been investigated, but the findings are limited by varying drug quality and sources, as well as the numerous and complicated components of formulas. Thus, it has been suggested that dietary inhibitors may be a solution for certain infections as an alternative approach[8]. Eradication of *H. pylori* has remained difficult for reasons that lie in the biology and environment of the organism. *H. pylori* populations colonize epithelial cells that line the antrum of the stomach and survive in the acidic environment[13]. Most anti-microbial agents are poorly secreted in the gastric mucosa because of the stomach environment[14], and the residues expressed on *H. pylori* enable specific binding to the mucus layers. Thus, CaG-NANA was excogitated to overcome the acidic environment of the stomach and have bactericidal activity by interrupting glycol-conjugation of *H. pylori* on the mucosa.

CaG-NANA, a compound of N-acetylneuraminic acid with calcium combined glycomacropeptide, demonstrated an anti-bacterial effect against *H. pylori*. We manufactured CaG-NANA as a dietary inhibitor against *H. pylori*; NANA was demonstrated as an *H. pylori* adhesion blocker[9,15,16], glycomacropeptide was used for modulating the acidic phase of the stomach, and calcium was inserted for improvement as functional food. NANA content in CaG-NANA was evaluated compared to S-NANA by HPLC, which confirmed its content and purity (Figure 1B). CaG-NANA at concentrations > 0.25% regulated the population of *H. pylori in vitro*. S-NANA also decreased the population of *H. pylori in vitro* (Figure 2D); however, S-NANA resulted in a severe weight loss *in vivo* (Figure 3B), which demonstrated that S-NANA treatment only had negative utility. Meanwhile, G-NANA and CaG-NANA had bactericidal and anti-adhesion effects on glyco-conjugation of the mucosa without toxicity. Bacterial attachment is mediated by outer membrane adhesions that bind to glycoconjugates present in the gastric mucus layer and lining the surface epithelium of

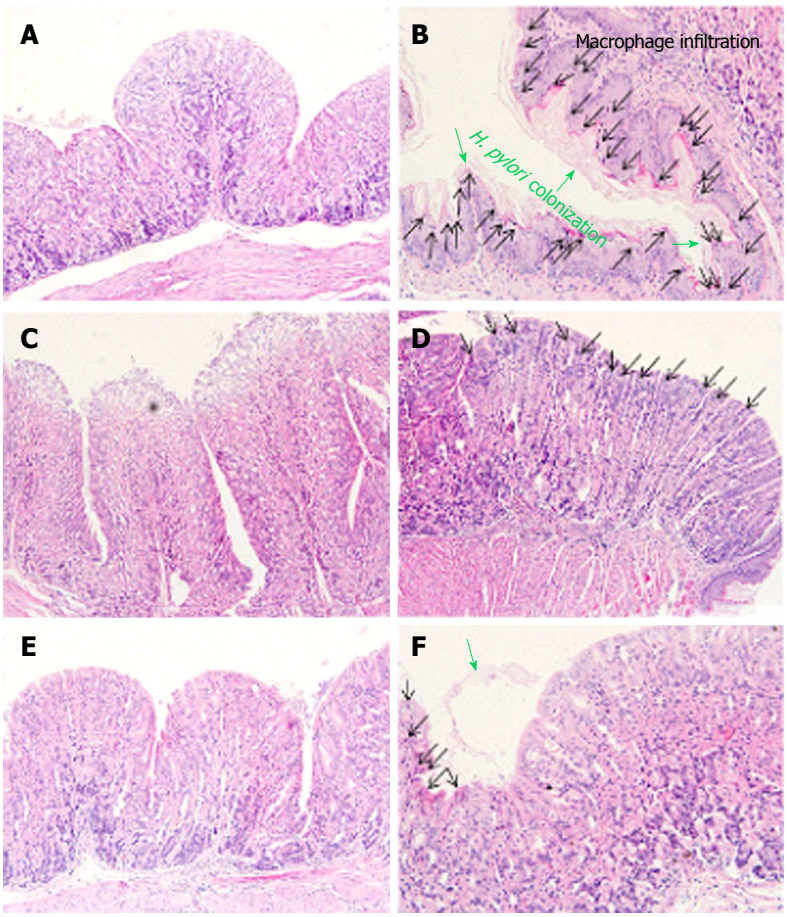

Figure 4 Histology of the gastric mucosa. Mouse stomachs were removed and underwent hematoxylin and eosin staining. *H. pylori* colonization and macrophage infiltration were observed under a light microscope. A: Normal group; B: *H. pylori* infected group; C: Antibiotics treated group; D: S-NANA treated group; E: G-NANA treated group; F: CaG-NANA treated group. S-NANA: Standard N-acetylneuraminic acid; G-NANA: Glycomacropeptide-N-acetylneuraminic acid; CaG-NANA: Calcium-glycomacropeptide-N-acetylneuraminic acid.

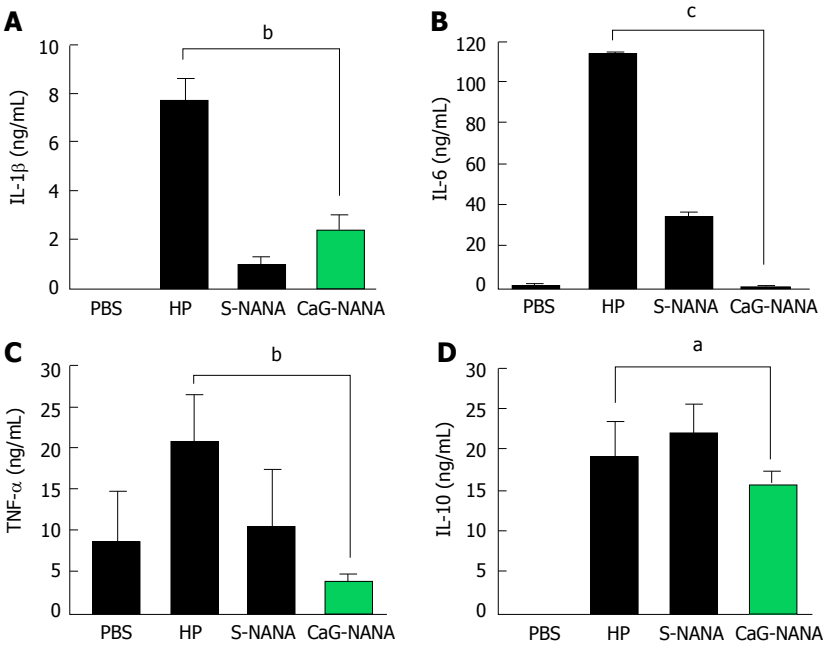

Figure 5 Expression levels of interleukine-1β, interleukine-6, tumor necrosis factor-α, and interleukine-10 in blood serum determined by enzyme-linked immunosorbent assay. The assays were performed according to the manufacturer's instructions and all experiments were repeated in triplicate. Statistical analysis was performed using Dunn's multiple comparison test with significance set at $^{a}P < 0.1$ and $^{b}P < 0.05$, $^{c}P < 0.01$. A: IL-1β; B: IL-6; C: TNF-α; D: IL-10. IL: Interleukine; TNF: Tumor necrosis factor; S-NANA: Standard N-acetylneuraminic acid; G-NANA: Glycomacropeptide-N-acetylneuraminic acid; CaG-NANA: Calcium-glycomacropeptide-N-acetylneuraminic acid.

the gastric mucosa[17].

H. pylori is also known to induce inflammatory response that includes the up-regulation of pro-inflammatory cytokines. CaG-NANA exerted a down-regulatory effect on pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α and IL-10. Reduction of pro-inflammatory cytokines induced by *H. pylori* is responsible for the recruitment of macrophages and neutrophils in the lamina propria. CaG-NANA exerted an antagonistic effect against *H. pylori*, which is an anti-microbial effect *via* different mechanisms from those of antibiotics.

This study suggests that it is possible to decrease the number of *H. pylori* or its activation in the stomach through a regular ingestion of N-acetylneuraminic acid containing glycomacropeptide as dietary products. Moreover, CaG-NANA is a complex compound which can be manufactured in large scale at a low cost under good manufacturing practice (GMP). In conclusion, the anti-*H. pylori* effects of CaG-NANA were confirmed both *in vitro* and *in vivo*, which provided experimental support for future human trials.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) has been reported to be associated with many gastrointestinal diseases such as chronic gastritis, peptic ulcers, gastric carcinoma, and mucosa-associated lymphoid tissue lymphoma. Until now, standard triple therapy consisting of a proton pump inhibitor and two broad-spectrum antibiotics, usually amoxicillin, clarithromycin or metronidazole, has been able to achieve eradication.

Research frontiers

The authors introduce a new N-acetylneuraminic acid (NANA) combined glycomacropeptide (GMP) which was made from milk serum hydrolysis protein powder as an anti-*H. pylori* agent. Wadstorm *et al* reported that NANA derivative had an adhesion potential to *H. pylori* lectins and Hirmo *et al* reported that milk glycoprotein had an anti-*H. pylori* effect. The authors designed a new material with NANA, GMP, and calcium (CaG-NANA), and anti-*H. pylori* activities of CaG-NANA were investigated both *in vitro* and *in vivo*.

Innovations and breakthroughs

The anti-*H. pylori* effects of CaG-NANA were confirmed both *in vitro* and *in vivo*, which provided experimental support for future human trials.

Applications

CaG-NANA is a complex compound which can be manufactured in a large scale at a low cost under GMP.

Peer-review

In the present paper, entitled "Anti-*Helicobacter pylori* effect of CaG-NANA, a new sialic acid derivative", Rhee *H et al* have investigated the effect of a compound named Cag-NANA in an animal model of *H. pylori*-associated gastritis and, *in vitro*, in bacterial cultures.

REFERENCES

1 **Simon PM**, Goode PL, Mobasseri A, Zopf D. Inhibition of Helicobacter pylori binding to gastrointestinal epithelial cells by sialic acid-containing oligosaccharides. *Infect Immun* 1997; **65**: 750-757 [PMID: 9009338]

2 **Logan RP**. Adherence of Helicobacter pylori. *Aliment Pharmacol Ther* 1996; **10** Suppl 1: 3-15 [PMID: 8730255 DOI: 10.1046/j.1365-2036.1996.22164001.x]

3 **Opekun AR**, El-Zaimaity HM, Osato MS, Gilger MA, Malaty HM, Terry M, Headon DR, Graham DY. Novel therapies for Helicobacter pylori infection. *Aliment Pharmacol Ther* 1999; **13**: 35-42 [PMID: 9892877 DOI: 10.1046/j.1365-2036.1999.00435.x]

4 **Kigasawa K**, Ohtani H. Decomposition and stabilization of drugs. XV. Structure and stability of aminoalkylesters. (7) (author's transl). *Yakugaku Zasshi* 1976; **96**: 6-11 [PMID: 943510]

5 **Yang JC**, Chien CT. A new approach for the prevention and treatment of Helicobacter pylori infection via upregulation of autophagy and downregulation of apoptosis. *Autophagy* 2009; **5**: 413-414 [PMID: 19197143]

6 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

7 **Caron TJ**, Scott KE, Fox JG, Hagen SJ. Tight junction disruption: Helicobacter pylori and dysregulation of the gastric mucosal barrier. *World J Gastroenterol* 2015; **21**: 11411-11427 [PMID: 26523106 DOI: 10.3748/wjg.v21.i40.11411]

8 **Burger O**, Ofek I, Tabak M, Weiss EI, Sharon N, Neeman I. A high molecular mass constituent of cranberry juice inhibits helicobacter pylori adhesion to human gastric mucus. *FEMS Immunol Med Microbiol* 2000; **29**: 295-301 [PMID: 11118911 DOI: 10.1111/j.1574-695X.2000.tb01537.x]

9 **Joffe M**. Epidemiology of occupational reproductive hazards: methodological aspects. *Rev Epidemiol Sante Publique* 1992; **40** Suppl 1: S17-S25 [PMID: 1626103 DOI: 10.1371/journal.ppat.0020110]

10 **Teneberg S**. The Multiple Carbohydrate Binding Specificities of Helicobacter pylori. *Top Curr Chem* 2009; **288**: 121-138 [PMID: 22328028 DOI: 10.1007/128_2008_14]

11 **Wadström T**, Hirmo S, Borén T. Biochemical aspects of Helicobacter pylori colonization of the human gastric mucosa. *Aliment Pharmacol Ther* 1996; **10** Suppl 1: 17-27 [PMID: 8730256 DOI: 10.1046/j.1365-2036.1996.22164002.x]

12 **Hirmo S**, Kelm S, Iwersen M, Hotta K, Goso Y, Ishihara K, Suguri T, Morita M, Wadström T, Schauer R. Inhibition of Helicobacter pylori sialic acid-specific haemagglutination by human gastrointestinal mucins and milk glycoproteins. *FEMS Immunol Med Microbiol* 1998; **20**: 275-281 [PMID: 9626932 DOI: 10.1111/j.1574-695X.1998.tb01137.x]

13 **Falk P**, Roth KA, Borén T, Westblom TU, Gordon JI, Normark S. An in vitro adherence assay reveals that Helicobacter pylori exhibits cell lineage-specific tropism in the human gastric epithelium. *Proc Natl Acad Sci USA* 1993; **90**: 2035-2039 [PMID: 8383333]

14 **Sakarya S**, Gunay N. Saccharomyces boulardii expresses neuraminidase activity selective for α2,3-linked sialic acid that decreases Helicobacter pylori adhesion to host cells. *APMIS* 2014; **122**: 941-950 [PMID: 24628732 DOI: 10.1111/apm.12237]

15 **Narod S**. Measles vaccination in Haiti. *N Engl J Med* 1986; **314**: 581-582 [PMID: 3945300 DOI: 10.1074/jbc.M113.513135]

16 **Salcedo J**, Barbera R, Matencio E, Alegría A, Lagarda MJ. Gangliosides and sialic acid effects upon newborn pathogenic bacteria adhesion: an in vitro study. *Food Chem* 2013; **136**: 726-734 [PMID: 23122120 DOI: 10.1016/j.foodchem.2012.08.078]

17 **Valkonen KH**, Wadström T, Moran AP. Identification of the N-acetylneuraminyllactose-specific laminin-binding protein of Helicobacter pylori. *Infect Immun* 1997; **65**: 916-923 [PMID: 9038297]

P- Reviewer: Ierardi E, Pellicano R **S- Editor**: Qi Y
L- Editor: Wang TQ **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com