J Antimicrob Chemother 2018; **73**: 1324–1329
doi:10.1093/jac/dky011 Advance Access publication 12 February 2018

Journal of Antimicrobial Chemotherapy

Gentamicin-intercalated smectite as a new therapeutic option for *Helicobacter pylori* eradication

Su Jin Jeong[1], Jie-Hyun Kim[2], Da Hyun Jung[2], Kyoung Hwa Lee[1], Soon Young Park[1], Yungoo Song[3], Il-Mo Kang[4] and Young Goo Song[1]*

[1]*Division of Infectious Disease, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea;* [2]*Division of Gastroenterology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea;* [3]*Department of Earth System Sciences, Yonsei University, Seoul, Republic of Korea;* [4]*Korea Institute of Geoscience and Mineral Resources, Daejeon, Republic of Korea*

*Corresponding author. Division of Infectious Disease, Department of Internal Medicine, Yonsei University College of Medicine, 211 Eonju-ro, Gangnam-gu, Seoul 06273, Korea. Tel: +82-2-2019-3319; Fax: +82-2-3463-3882; E-mail: imfell@yuhs.ac

Received 28 September 2017; returned 28 November 2017; revised 20 December 2017; accepted 2 January 2018

Objectives: Novel antibacterial strategies against *Helicobacter pylori* are needed because *H. pylori* strains are acquiring resistance to antibiotics. We evaluated the efficacy of gentamicin-intercalated smectite hybrid (S-GEN)-based treatment regimens in a murine model of *H. pylori* infection.

Methods: Two groups of 10 rats were administered either smectite or S-GEN to measure coverage of the gastric mucosa. To evaluate anti-*H. pylori* efficacy, mice were divided into eight groups of 10 mice each given different treatments, and *H. pylori* eradication was assessed by a *Campylobacter*-like organism (CLO) test and *H. pylori* PCR of the gastric mucosa, and *H. pylori* antigen and *H. pylori* PCR analysis of mouse faeces. The levels of proinflammatory cytokines were examined.

Results: S-GEN was retained in the gastric mucosal layer with a >60% distribution ratio for up to 1 h, and the S-GEN-based triple regimen decreased bacterial burden *in vivo* compared with that of untreated mice or mice treated with other regimens. The cure rates in the CLO test and *H. pylori* PCR from gastric mucosa were 70%, 60%, 80%, 50%, 60% and 60% in Groups III–VIII, respectively. Those for *H. pylori* PCR in the faeces of mice were 90% and 100% in Group III with standard therapy and Group V with triple therapy including S-GEN, respectively. S-GEN triple therapy also reduced the levels of proinflammatory cytokines.

Conclusions: These results suggest that S-GEN is a promising and effective therapeutic agent for the treatment of *H. pylori* infection.

Introduction

Helicobacter pylori is one of the most predominant bacterial pathogens in humans, colonizing the stomachs of nearly half of the global population. *H. pylori* infection is responsible for most cases of inflammatory gastritis, peptic ulcer disease and gastric cancer in humans.[1] Various attempts to develop preventive vaccines against *H. pylori* have failed. Upon diagnosis, *H. pylori* infection is treated with conventional antibiotic regimens. However, the success rate of these treatments has been compromised by the drastic increase in antimicrobial-resistant *H. pylori* strains.[2] The prevalence of *H. pylori* resistance to metronidazole, which is a key component of the triple-therapy regimen, has increased to ∼40% in developed countries, with a higher prevalence of ∼90% in developing countries.[3]

The prevalence of MDR *H. pylori* strains is rapidly increasing; hence, there is an alarming need to develop alternative and effective antimicrobial agents. Among the promising candidates are aminoglycosides, of which gentamicin, tobramycin and netilmicin show high activity against *H. pylori*, with an MIC required to inhibit 50% of bacteria (MIC_{50}) of 0.125 mg/L.[4] However, aminoglycosides are polar, water-soluble compounds with very poor intestinal membrane permeability, resulting in low oral bioavailability.[5,6] Therefore, intravenous or intramuscular injections are required, increasing the risk of adverse effects, such as nephrotoxicity and ototoxicity, and increasing hospitalization-associated costs. Thus, there are limitations when developing aminoglycosides as new anti-*H. pylori* treatments.

Smectite clay, a type of layered aluminosilicate, is composed of tetrahedral sheets of SiO_4 units and octahedral sheets of Al^{3+} ions.[7] It possesses hydrophilicity, high dispersibility in water and cation exchange capacity.[8] Therefore, smectite clays can encapsulate various protonated and hydrophilic organic molecules

into the interlayer space of the plane, making them good carriers for hydrophilic drugs.[9] In this study, a gentamicin-intercalated smectite hybrid (S-GEN) was successfully synthesized. This should prolong gentamicin's gastric residence time, generating a local therapeutic effect against *H. pylori* without adverse effects.

Thus, the study was aimed at comparing the therapeutic efficacy of S-GEN and other regimens, including standard triple therapy, using a mouse model of *H. pylori* infection. To assess host response to S-GEN treatment, we examined the levels of proinflammatory cytokines, including IL-8 and TNF-α, during the treatment.

Methods

Intercalation of gentamicin

Gentamicin solution (2 mg/mL) was prepared using United States Pharmacopeia (USP)-grade gentamicin sulphate produced by Bio Basic Inc. Ca-smectite was prepared by purifying bentonite from the Gampo area in Korea. S-GEN was produced by mixing 250 mL of gentamicin solution per gram of Ca-smectite, and stirring vigorously for 24 h. After mixing, the hybrid solution was dialysed with 5 L of distilled water for ∼8 h at 50°C, which was repeated three or four times until sulphate ions could not be detected with $PbCl_2$. The hybrid powder was obtained by freeze-drying the dialysed hybrid solution for 2–3 days. The amount of gentamicin released from the hybrid was determined by a batch-release test, in which 25 mL of pH 1.2 solution was added repeatedly to the same 100 mg of hybrid powder. Gentamicin concentration from the supernatant was measured using LC–MS. LC analyses were performed using the Thermo Scientific ICS system. MS analysis was performed using a Thermo Scientific MSQ Plus single-quadrupole mass spectrometer with electrospray ionization. The total amount of gentamicin released within 1 h was determined to be ∼5.0 mg per 100 mg of the hybrid.

Ethics

The Institutional Animal Care and Use Committee at the National Center of Efficacy Evaluation for the Development of Health Products Targeting Digestive Disorders, Incheon, Korea, approved the animal procedures conducted on rats.

Mouse experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Wonkwang University College of Medicine. Mice were fed a standard laboratory diet with water *ad libitum* and treated according to the guidelines and regulations for the use and care of animals of Wonkwang University, Iksan, Korea.

Covering the gastric mucosa in rats

Seven-week-old male Sprague–Dawley rats (weighing 220 g ± 20%) were purchased from Samtako Ltd., Osan, Korea, to test the coverage of the gastric mucosa. The rats were fasted for 24 h before experiments. Two groups of 10 rats were administered either smectite or S-GEN at 10 mL/kg (150 mg/kg) and euthanized after 1 h. For analysing the covering efficiency of the gastric mucosa, their stomachs were excised, cut along the greater curvature, and pinned. The gastric distribution ratio was calculated using the following formula:

$$\text{Gastric distribution ratio (\%)} = \frac{\text{Gastric distribution area (cm}^2\text{)}}{\text{Gastric total area (cm}^2\text{)}} \times 100$$

The ratio and area were analysed using the Leica Application Suite V4 (Leica Microsystems Ltd., Korea). The gastric distribution ratio was expressed as mean (± SD).

Anti-H. pylori efficacy in vivo

Inoculation of experimental animals

Four-week-old male C57BL/6 mice were purchased from Japan SLC, Inc., Shizuoka, Japan, for anti-*H. pylori* assessments. Mice were 5 weeks of age and weighed 18–20 g at the start of the experiments.

H. pylori SS1 was used for inoculation. The bacteria were maintained on Brucella Blood Agar (Merck, Germany) at 37°C under microaerobic conditions (10% CO_2, 85% N_2 and 5% O_2) for 72 h. For the *in vivo* anti-*H. pylori* assessment, 80 mice were acclimatized for 1 week before the experiment. After acclimatization, the animals were fasted for 12 h, and 70 were infected with 0.5 mL of 2.0×10^9 cfu/mL *H. pylori* suspension, administered intragastrically through oral gavage every 48 h, three times per week. The inoculation day was considered day 0 and subsequent days as days 1–21. A group of uninfected mice, serving as the normal control group, received an equivalent volume of PBS and distilled water.

Distribution of animals

The mice were distributed into the following eight groups of 10 animals and allowed to rest for 1 week after the last inoculation: Group I, normal group with uninfected mice; Group II, no-treatment control group, received distilled water; Group III, treated with amoxicillin (14.25 mg/kg), clarithromycin (7.15 mg/kg) and a proton pump inhibitor (PPI, omeprazole was used in all groups that received a PPI, 400 μmol/kg), and served as a positive control group; Group IV, treated with amoxicillin (14.25 mg/kg), gentamicin (4 mg/kg) and PPI (400 μmol/kg); Group V, treated with amoxicillin (14.25 mg/kg), S-GEN (78 mg/kg) and PPI (400 μmol/kg); Group VI, treated with gentamicin (4 mg/kg) and PPI (400 μmol/kg); Group VII, treated with S-GEN (78 mg/kg) and PPI (400 μmol/kg); and Group VIII, treated with amoxicillin (14.25 mg/kg) and PPI (400 μmol/kg). Treatments were administered orally once daily for 7 consecutive days. To confirm the *H. pylori* serological status of the infected mice, *H. pylori* immunoglobulin G (IgG) levels were determined with an ELISA kit (Cusabio Biotech Co., USA) before treatment.

Bacterial identification

Twelve hours after the last administration, mice were euthanized and stomachs were removed from their abdominal cavities. The gastric mucosa from the pylorus was biopsied for the *Campylobacter*-like organism (CLO) test and PCR for *H. pylori*. Additionally, 0.5 g of faeces per mouse was collected from the rectum and colon, suspended in the same volume of distilled water and filtered for *H. pylori* antigen (Ag) detection and *H. pylori* PCR in faeces.

CLO test

The gastric mucosal samples of the pyloric region were assayed with CLO kits (Asan Pharmaceutical Co., Seoul, Korea) and incubated at 37°C for 12 h to examine urease activity. The reaction (colour change) was deemed negative when bright yellow or positive when dark red. The reaction score was graded from 0 to 3 with 0 = no colour change, 1 = bright red, 2 = light purple and 3 = dark red.

H. pylori PCR of the gastric mucosa

H. pylori DNA was prepared using the previously described bead beater/phenol extraction method.[10] A bacterial suspension was placed in a 2.0 mL screw-cap microcentrifuge tube filled with 200 μL (paced volume) of glass beads (diameter, 0.1 mm; Biospec Products, Bartlesville, OK, USA) and 200 μL of phenol:chloroform:isoamyl alcohol (50:49:1). The tube was oscillated on a Mini-Bead Beater (Biospec Products) for 30 s and centrifuged (12 000 **g** for 15 min) to separate the phases. The aqueous phase was subsequently transferred

into another clean tube, and 10 μL of 3 M sodium acetate and 250 μL of ice-cold absolute ethanol were added. To precipitate DNA, the mixture was kept at −20°C for 10 min. The harvested DNA pellets were dissolved in 60 μL of Tris–EDTA buffer (pH 8.0) and used as template DNA for PCR. PCR was performed using AccuPower PCR Premix (Bioneer, Daejeon, Korea). After an initial denaturation/activation step (95°C for 5 min), DNA (50 ng) was amplified in a 20 μL volume for 35 cycles of denaturation (94°C for 60 s), annealing (62°C for 60 s) and extension (72°C for 90 s), using the following primers: *H. pylori*-specific *ureA* and *ureC*, sense 5′-TGATGCTCCACTACGCTGGA-3′ and antisense 5′-GGGTATGCACGGTTACGAGT-3′ (expected product 265 bp),[11] and GAPDH, sense 5′-TGGGGTGATGCTGGTGCTG-AG-3′ and antisense 5′-GGTTTCTC CAGGCGGCATGTC-3′ (expected product 497 bp).[12] The PCR products were analysed by electrophoresis in 1.5% agarose gels.

H. pylori antigen in mouse faeces

The *H. pylori* antigen was evaluated using the commercially available SD Bioline *H. pylori* Ag kit (Standard Diagnostics, Inc.) according to the manufacturer's instructions. Specimens (250 mg) were incubated with a diluent solution at room temperature for 30 min and then 100 μL was placed in the *H. pylori* Ag examination device. The results were checked ∼15 min later. A single red line indicated a negative and a double red line indicated a positive *H. pylori* result.[13]

H. pylori PCR of mouse faeces

Genomic DNA from stool specimens was extracted using the AccuPrep Stool DNA Extraction Kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions.[14] A set of primers (sense 5′-TGATGCT CCACTACGCTGGA-3′ and antisense, 5′-GGGTATGCACGGTTACGAGT-3′) was used to amplify *H. pylori*-specific *ureA* and *ureC* (265 bp).[11] Template DNA (50 ng) and 20 pmol of each primer were added to a PCR mixture tube containing 1 U of *Taq* DNA polymerase, 250 μM each deoxynucleoside triphosphate, 50 mM Tris–HCl (pH 8.3), 40 mM KCl, 1.5 mM $MgCl_2$ and the gel loading dye. The volume was adjusted to 20 μL with distilled water. After initial denaturation at 95°C for 5 min, the reaction mixture was subjected to 35 amplification cycles (60 s at 94°C, 60 s at 62°C and 90 s at 72°C), followed by a 10 min extension at 72°C (GeneAmp 9700, Perkin Elmer, USA). The PCR products were electrophoresed on 1.5% agarose gel.[15]

Quantification of inflammatory cytokines

Plasma was obtained for IL-8 and TNF-α assays on day 21 by the insertion of a heparinized microhaematocrit tube into the ophthalmic venous plexus of the mice. Plasma IL-8 and TNF-α levels were measured using mouse ELISA kits (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

Data are presented as means ± SE, and groups were compared using the non-parametric Mann–Whitney test. We obtained 95% CIs for the detection rate using the MINITAB statistical software program (Minitab Inc., PA, USA). If the 95% CIs of two values did not overlap, they were considered significantly different. A P value <0.05 was considered statistically significant. Results were analysed using the Statistics Package for Social Science (SPSS 12.0 for Windows; SPSS Inc., Chicago, IL, USA).

Results

Coverage of the gastric mucous layer in rats

We chose the experimental conditions of 10 mL/kg (150 mg/kg) and euthanized the experimental rats 1 h after oral administration, considering the drug release time from the hybrid. S-GEN showed a 60.2% (± 14.3%) distribution ratio.

In vivo anti-H. pylori assessment

To evaluate the *in vivo* therapeutic efficacy of S-GEN against *H. pylori*, we established the *H. pylori* infection model (Figure 1). Furthermore, to confirm *H. pylori* infection, *H. pylori* IgG levels were determined before and after treatment (Table 1).

CLO test and H. pylori PCR of gastric mucosa

Repeated intragastric inoculation of *H. pylori* in mice produced a positive reaction (red colour) in the CLO test of the gastric mucosa (Table 2). The cure rates for gastric mucosa (100 − positive reactions) were 70%, 60%, 80%, 50%, 60% and 60% in Groups III–VIII, respectively. The Group V CLO score was the lowest among the *H. pylori*-infected groups and was significantly lower than that of Group II.

The PCR products of *H. pylori*-specific *ureA* and *ureC* (265 bp) were electrophoresed on 1.5% agarose gel, and visualized (Figure S1, available as Supplementary data at *JAC* Online). The cure rates were identical to those determined by the CLO test.

H. pylori antigen and PCR in faeces of mice

We used a stool antigen kit to detect *H. pylori* in faeces. We consistently observed positive results in Group II and negative results in the other groups.

H. pylori PCR was conducted to evaluate therapeutic effects in *H. pylori*-infected mice (Table 3). The cure rates were 90% and 100%, respectively, in Group III (standard therapy) and Group V (therapy with amoxicillin/S-GEN/PPI).

Quantification of inflammatory cytokines

To determine whether anti-*H. pylori* treatment, including S-GEN, influences the production of cytokines, plasma concentrations of inflammatory cytokines were measured in the mice (Table 4). The levels of IL-8 and TNF-α in the treatment groups were significantly lower than those in Group II (Table 4). The plasma levels of IL-8 and TNF-α in Group V were the lowest among treatment groups.

Discussion

H. pylori is a Gram-negative microaerophilic helical bacillus that affects the gastric mucosa and can be found attached to epithelial cells of the human stomach.[16] Approximately 50% of the world population is *H. pylori* positive, with the developing countries having a prevalence of 80%–90% and industrialized countries having a prevalence of 35%–40%.[17] Quadruple regimens are used as salvage therapy when the standard, triple therapy regimen fails.[18] Drug-resistant *H. pylori* strains are the most common cause of treatment failure.[19] In the present study, we evaluated the anti-*H. pylori* efficacy of S-GEN in a mouse model. Our results demonstrated significantly improved antimicrobial efficacy of S-GEN in reducing the *H. pylori* load in mouse stomachs compared with that of other treatment regimens, including triple therapy, the current worldwide standard for *H. pylori* treatment.

Smectite is a phyllosilicate mineral composed of two sheets of $(Si,Al)O_4$ tetrahedra and one sheet of $(Mg,Fe,Al)O_4(OH)_2$ edge-sharing octahedra, which form a sandwich-shaped structure where one octahedral sheet is inserted between two tetrahedral sheets. Isomorphous substitutions of Al for Si in the tetrahedral

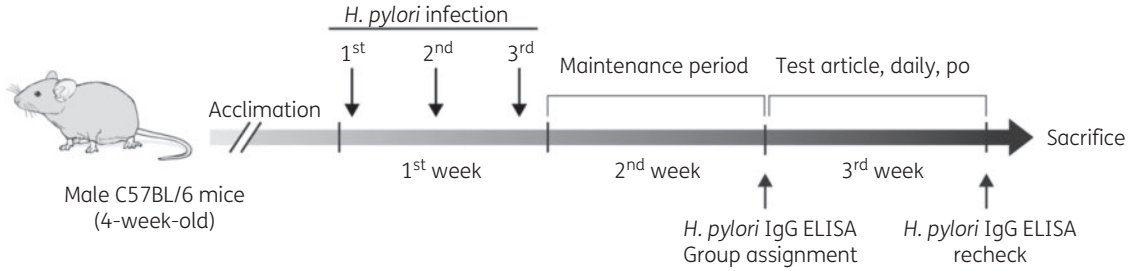

Figure 1. Anti-*H. pylori* efficacy *in vivo*. The study protocol, including *H. pylori* inoculation and infection development in C57BL/6 mice, followed by the treatments.

Table 1. Plasma concentration of *H. pylori* IgG in each group

	Inoculation			*H. pylori* IgG concentration	
Group	*H. pylori* infection	treatment	*N*	before treatment	after treatment
I	no	DW	10	0.26±0.01	0.25±0.00**
II	yes	DW	10		0.74±0.01
III	yes	AMX + CLR + PPI	10		0.33±0.01**
IV	yes	AMX + GEN + PPI	10		0.35±0.01**
V	yes	AMX + S-GEN + PPI	10	0.58±0.05[a]*	0.32±0.01**
VI	yes	GEN + PPI	10		0.40±0.01**
VII	yes	S-GEN + PPI	10		0.34±0.01**
VIII	yes	AMX + PPI	10		0.35±0.01**

DW, distilled water; AMX, amoxicillin; CLR, clarithromycin; GEN, gentamicin.
[a]Data are expressed as means ± SE of 70 infected mice (Groups II–VIII).
*Significantly different from Group I ($P < 0.01$).
**Significantly different from Group II ($P < 0.01$).

Table 2. Results of CLO test with gastric mucosa after treatment

Group[a]	Percentage of animals negative by CLO test (95% CI[b])	CLO score
I	100 (72.2–100.0)	0.0±0.0
II	0 (0.0–27.6)	3.0±0.0
III	70 (39.7–89.2)*	0.9±1.5*
IV	60 (31.2–83.1)*	1.2±1.6*
V	80 (49.0–94.3)*	0.6±1.3*
VI	50 (23.7–76.3)	1.5±1.6
VII	60 (31.2–83.1)*	1.2±1.6*
VIII	60 (31.2–83.1)*	1.2±1.6*

[a]Each group consisted of 10 mice.
[b]Incidence percentage (95% CI) was calculated with the MiniTab statistics program.
*Significantly different from Group II ($P < 0.05$).

Table 3. PCR analysis of *H. pylori* in faeces after treatment

Group[a]	Percentage of animals negative by faeces PCR (95% CI[b])
I	100 (72.2–100.0)
II	0 (0–27.6)
III	90 (60.0–98.2)*
IV	80 (49.0–94.3)*
V	100 (72.2–100.0)*
VI	70 (39.7–89.2)*
VII	80 (49.0–94.3)*
VIII	70 (39.7–89.2)*

[a]Each group consisted of 10 mice.
[b]Incidence percentage (95% CI) was calculated with the MiniTab statistics program.
*Statistically significantly different from Group II ($P < 0.05$).

sheet and Mg for Al and Fe^{3+} in the octahedral sheet induce a negative charge that causes the interlayer to be an active cation-exchange site, indicating that smectite could be used as a drug carrier.[7,20] Therefore, smectite's use as a drug delivery vehicle has attracted great interest recently, and various studies have reported different drug-intercalated smectite hybrids for controlled delivery and release of drugs, including donepezil,[21] lincomycin,[22] chlorhexidine acetate[23] and tetracycline.[24,25]

Evaluation of the *in vitro* activity of some aminoglycosides found that they might serve as alternative agents in combination therapy regimens.[4] Of the aminoglycosides tested, the most active were gentamicin, tobramycin and netilmicin, with MIC_{90} and MIC_{50}

Table 4. Plasma concentrations of IL-8 and TNF-α

Group[a]	IL-8 concentration (μg/mL)	TNF-α concentration (μg/mL)
I	3.73±0.82*	16.14±4.99*
II	7.71±0.66	44.43±6.23
III	4.12±0.45*	23.59±0.48**
IV	3.98±0.21*	23.20±2.52**
V	3.57±0.38*	17.65±3.21**
VI	4.24±0.42*	24.30±1.84**
VII	4.08±0.25*	18.76±1.33**
VIII	4.52±0.36*	22.13±3.59**

Data are expressed as mean ± SE for 10 mice per group (μg/mL).
[a]Each group consisted of 10 mice.
*Significantly different from Group II ($P < 0.05$).
**Significantly different from Group II ($P < 0.01$).

values of 0.25–0.5 and 0.125–1.00 mg/L, respectively.[4] However, aminoglycosides are very poorly absorbed when administered orally, and must be given parenterally for systemic infections.[26] Furthermore, the major limitation of the clinical use of aminoglycosides continues to be concerns about nephrotoxicity and ototoxicity.[27] Evidence from previous reports has demonstrated a correlation between the nephrotoxic effects of aminoglycosides and the accumulation of these drugs in the kidney cortex.[28,29] Therefore, we developed S-GEN and evaluated whether it could be effective for the treatment of *H. pylori* infection.

H. pylori mainly resides within the layer of adherent mucus close to the epithelial surface.[30] Therefore, for effective treatment, S-GEN must cross the mucus layer and be retained on the stomach wall. In this study, the S-GEN was well distributed on the distal gastric wall; the observed 60.2% presence of S-GEN indicated effective S-GEN retention for up to 1 h. This result suggests that S-GEN could be used for direct eradication of *H. pylori*.

In the results of the CLO tests and *H. pylori* PCR with gastric mucosa after treatment, Group V had the highest cure rate (80%) among the treated groups. Furthermore, the Group V CLO score was the lowest score among treatment groups (0.6±1.3). Anti-*H. pylori* efficacy was evident in Group V. In the case of the *H. pylori* PCR test with faeces after treatment for *H. pylori* infection, negative findings were consistently observed in Group V only. Therefore, the superior anti-*H. pylori* activity conferred by the S-GEN triple therapy can be explained by its direct killing effect and prolonged retention in the gastric mucosa compared with that of the other regimens of conventional antibiotics.

Interestingly, S-GEN-treated mice had significantly reduced *H. pylori*-induced proinflammatory cytokines (IL-8 and TNF-α) compared with *H. pylori*-infected but untreated mice. IL-8 attracts neutrophils, thereby promoting inflammation,[31] and TNF-α, together with IL-1β, induces gastrin secretion, suggesting a role for these cytokines in *H. pylori*-induced hypergastrinaemia and inflammatory responses.[32,33] The immune response to *H. pylori* contributes to disease pathogenesis.[34] Thus, reduction of the proinflammatory response, as observed here, is expected to reduce the inflammatory reaction responsible for perpetuating tissue injury. Upon S-GEN treatment, an early clearance of colonization and better anti-*H. pylori* effectiveness than that achieved with other regimens could reduce gastric inflammation. However, immunomodulatory properties of smectite itself in the tissue injury should be confirmed by further research.

The current study has a limitation that needs to be addressed. Tissue toxicity was not evaluated, limiting the information regarding the effect of S-GEN on gastric mucosa. In the present study, treatment with S-GEN had no effect on mouse body weight. The safety of smectite by oral administration has been demonstrated, and gentamicin was not absorbed through the gastric mucosa. However, the safety of S-GEN with regard to the mucosal layer should be confirmed by additional studies.

We demonstrated prolonged retention of S-GEN in the gastric mucosal layer, and potent antimicrobial activity of S-GEN against *H. pylori*. S-GEN treatment decreased the bacterial burden *in vivo*, compared with untreated mice or mice treated with double or triple therapy including PPI. In addition to its direct killing effect, S-GEN inhibited proinflammatory cytokine production to help reduce inflammatory responses. Overall, our data suggest that S-GEN is a promising candidate as an anti-*H. pylori* agent.

Acknowledgements

We thank Dong-Su Jang, MFA (Medical Illustrator, Medical Research Support Section, Yonsei University College of Medicine, Seoul, Korea) for his help with the illustrations.

Funding

This work was supported by the Basic Research Project (Study No. 16-3220) of the Korea Institute of Geoscience and Mineral Resources funded by the Ministry of Science, Information and Communication Technology and Future Planning of Korea.

Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online.

References

1 Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860–7.

2 Rimbara E, Fischbach LA, Graham DY. Optimal therapy for *Helicobacter pylori* infections. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 79–88.

3 Kaakoush NO, Asencio C, Mégraud F *et al*. A redox basis for metronidazole resistance in *Helicobacter pylori*. *Antimicrob Agents Chemother* 2009; **53**: 1884–91.

4 Brenciaglia MI, Fornara AM, Scaltrito MM *et al*. Activity of amoxicillin, metronidazole, bismuth salicylate and six aminoglycosides against *Helicobacter pylori*. *J Chemother* 1996; **8**: 52–4.

5 Cox CE. Gentamicin. *Med Clin North Am* 1970; **54**: 1305–15.

6 Recchia J, Lurantos MH, Amsden JA *et al*. A semisynthetic *Quillaja* saponin as a drug delivery agent for aminoglycoside antibiotics. *Pharm Res* 1995; **12**: 1917–23.

7 Thomas F, Michot LJ, Vantelon D *et al*. Layer charge and electrophoretic mobility of smectites. *Colloids Surf A Physicochem Eng Asp* 1999; **159**: 351–8.

8 Lin F-H, Lee Y-H, Jian C-H *et al*. A study of purified montmorillonite intercalated with 5-fluorouracil as drug carrier. *Biomaterials* 2002; **23**: 1981–7.

9 Yang I-K, Hu C-C. Preparation and rheological characterization of poly(n-butyl methacrylate)/montmorillonite composites. *Eur Polym J* 2006; **42**: 402–9.

10 Kim B-J, Lee S-H, Lyu M-A *et al*. Identification of mycobacterial species by comparative sequence analysis of the RNA polymerase gene (rpoB). *J Clin Microbiol* 1999; **37**: 1714–20.

11 Kim YB, Kim ST, Lee SW *et al*. The influence of number of gastroscopic biopsy specimens on follow-up *Campylobacter*-like organism (CLO) test. *Korean J Gastroenterol* 2000; **35**: 422–8.

12 Kundu P, Mukhopadhyay AK, Patra R *et al*. Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by *Helicobacter pylori* infection. *J Biol Chem* 2006; **281**: 34651–62.

13 Moon D-I, Shin E-H, Oh H-G *et al*. Usefulness of a *Helicobacter pylori* stool antigen test for diagnosing *H. pylori* infected C57BL/6 mice. *Lab Anim Res* 2013; **29**: 27–32.

14 Lee J-U, Jung K, Kim O. Absence of vertical transmission of *Helicobacter pylori* in an experimental murine model. *J Vet Sci* 2006; **7**: 225–8.

15 Lee H-A, Park Y-S, Kim O. Prevalence of *Helicobacter* species in feces of dogs using polymerase chain reaction analysis. *Lab Anim Res* 2007; **23**: 339–44.

16 Ndip R, Malange A, Akoachere J *et al*. *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limbe health districts of Cameroon: a pilot study. *Trop Med Int Health* 2004; **9**: 1036–40.

17 Lacy BE, Rosemore J. *Helicobacter pylori*: ulcers and more: the beginning of an era. *J Nutr* 2001; **131**: 2789S–93S.

18 Hoffman JS, Cave DR. Treatment of *Helicobacter pylori*. *Curr Opin Gastroenterol* 2001; **17**: 30–4.

19 Broutet N, Tchamgoue S, Pereira E *et al*. Risk factors for failure of *Helicobacter pylori* therapy—results of an individual data analysis of 2751 patients. *Aliment Pharmacol Ther* 2003; **17**: 99–109.

20 Swartzen-Allen SL, Matijevic E. Surface and colloid chemistry of clays. *Chem Rev* 1974; **74**: 385–400.

21 Park JK, Choy YB, Oh J-M *et al*. Controlled release of donepezil intercalated in smectite clays. *Int J Pharm* 2008; **359**: 198–204.

22 Wang C, Ding Y, Teppen BJ *et al*. Role of interlayer hydration in lincomycin sorption by smectite clays. *Environ Sci Technol* 2009; **43**: 6171–6.

23 Meng N, Zhou N-L, Zhang S-Q *et al*. Controlled release and antibacterial activity chlorhexidine acetate (CA) intercalated in montmorillonite. *Int J Pharm* 2009; **382**: 45–9.

24 Ghadiri M, Hau H, Chrzanowski W *et al*. Laponite clay as a carrier for in situ delivery of tetracycline. *RSC Adv* 2013; **3**: 20193–201.

25 Li Z, Chang P-H, Jean J-S *et al*. Interaction between tetracycline and smectite in aqueous solution. *J Colloid Interface Sci* 2010; **341**: 311–9.

26 Turnidge J. Pharmacodynamics and dosing of aminoglycosides. *Infect Dis Clin North Am* 2003; **17**: 503–28.

27 Rybak MJ, Abate BJ, Kang SL *et al*. Prospective evaluation of the effect of an aminoglycoside dosing regimen on rates of observed nephrotoxicity and ototoxicity. *Antimicrob Agents Chemother* 1999; **43**: 1549–55.

28 De Broe ME, Verbist L, Verpooten GA. Influence of dosage schedule on renal cortical accumulation of amikacin and tobramycin in man. *J Antimicrob Chemother* 1991; **27**: 41–7.

29 Mattie H, Craig W, Pechere J. Determinants of efficacy and toxicity of aminoglycosides. *J Antimicrob Chemother* 1989; **24**: 281–93.

30 Schreiber S, Konradt M, Groll C *et al*. The spatial orientation of *Helicobacter pylori* in the gastric mucus. *Proc Natl Acad Sci USA* 2004; **101**: 5024–9.

31 Sebkova L, Pellicanò A, Monteleone G *et al*. Extracellular signal-regulated protein kinase mediates interleukin 17 (IL-17)-induced IL-8 secretion in *Helicobacter pylori*-infected human gastric epithelial cells. *Infect Immun* 2004; **72**: 5019–26.

32 Weigert N, Schaffer K, Schusdziarra V *et al*. Gastrin secretion from primary cultures of rabbit antral G cells: stimulation by inflammatory cytokines. *Gastroenterology* 1996; **110**: 147–54.

33 Noah L, Bosma N, Jansen J. Mucosal tumor necrosis factor-α, interleukin-1β, and interleukin-8 production in patients with Helicobacter *pylori*. *Scand J Gastroenterol* 1994; **29**: 425–9.

34 Suarez G, Reyes VE, Beswick EJ. Immune response to *H. pylori*. *World J Gastroenterol* 2006; **12**: 5593.