Selective killing of Helicobacter pylori with pH-responsive helix–coil conformation transitionable antimicrobial polypeptides

Menghua Xiong1,a, Yan Bao1,b, Xin Xu1,c, Hua Wang1, Zhiyuan Han2, Zhiyu Wang3, Yeqing Liu4, Songyun Huang5, Ziyuan Song6, Jining Chen6, Richard M. Peek Jr.f, Lichen Yin1,c, Lin-Feng Chen1,b,2, and Jianjun Cheng1,a,c,d,g,h,i,j,2

Departments...affiliated institutions

Edited by Alexander M. Klibanov, Massachusetts Institute of Technology, Cambridge, MA, and approved October 20, 2017 (received for review June 9, 2017)

Current clinical treatment of Helicobacter pylori infection, the main etiological factor in the development of gastritis, gastric ulcers, and gastric carcinoma, requires a combination of at least two antibiotics and one proton pump inhibitor. However, such triple therapy suffers from progressively decreased therapeutic efficacy due to the drug resistance and undesired killing of the commensal bacteria due to poor selectivity. Here, we report the development of antimicrobial polypeptide-based monotherapy, which can specifically kill H. pylori under acidic pH in the stomach while inducing minimal toxicity to commensal bacteria under physiological pH. Specifically, we designed a class of pH-sensitive, helix–coil conformation transitionable antimicrobial polypeptides (HCT-AMPs) (PGA)m−f(PHLG-MHH)d, bearing randomly distributed negatively charged glutamic acid and positively charged poly-(N-methyldeethylammonium)hexyl-glutamate (PHLG-MHH) residues. The HCT-AMPs showed unappreciable toxicity at physiological pH when they adopted random coiled conformation. Under acidic condition in the stomach, they transformed to the helical structure and exhibited potent antibacterial activity against H. pylori, including clinically isolated drug-resistant strains. After oral gavage, the HCT-AMPs afforded comparable H. pylori killing efficacy to the triple-therapy approach while inducing minimal toxicity against normal tissues and commensal bacteria, in comparison with the remarkable killing of commensal bacteria by 65% and 86% in the ileal contents and feces, respectively, following triple therapy. This strategy renders an effective approach to specifically target and kill H. pylori in the stomach while not harming the commensal bacteria/normal tissues.

Clinical treatment of Helicobacter pylori using combination therapy is greatly challenged by the undesired killing of commensal bacteria and progressive development of drug resistance. To address these issues, we developed pH-sensitive, helix–coil conformation transitionable, antimicrobial polypeptides as a single therapeutic agent to selectively kill H. pylori under acidic condition in the stomach with minimal toxicity to commensal bacteria and diminished drug resistance. Through the control of the secondary structure transition, the polypeptides showed unappreciable toxicity to commensal bacteria and tissues at physiological pH when they adopted random coiled conformation, while the restoration of helical structure in the acidic stomach allowed the polypeptide to regain membrane disruptive capability to effectively and selectively kill H. pylori, including drug-resistant strains.


The authors declare no conflict of interest.

Significance

This article is a PNAS Direct Submission. Published under the PNAS license.

1M.X., Y.B., and X.X. contributed equally to this work.
2To whom correspondence may be addressed. Email: lichen@illinois.edu, or jianjun@illinois.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1710408114/-/DCSupplemental.

10.1073/pnas.1710408114

www.pnas.org/cgi/doi/10.1073/pnas.1710408114

PNAS | November 28, 2017 | vol. 114 | no. 48 | 12675–12680
activity that are associated with their helical structure (44, 45). While these RA polypeptides afford several advantages over conventional AMPs, such as simplicity in design and stability against proteases (44), they lack the capability of differentiating pathogenic bacteria from commensal bacteria, which will cause nonspecific killing of commensal bacteria when applied for anti- \textit{H. pylori} therapy.

Considering that \textit{H. pylori} thrive in the stomach under unique acidic condition (with a mean gastric pH of \(\sim 2\) in human) while commensal bacteria reside in the intestine with relatively neutral pH (6–8) (46–48), we herein developed a class of pH-sensitive, helix-coil conformation transitional antimicrobial polypeptides (HCT-AMPs) (PGA)\(_{m-r}^{-}(\text{PHLG-MHH})_{n}\) with both anionic groups (glutamic acid and cationic groups (tryptophan)), ultimately leading to minimal toxicity to intestinal commensal bacteria, while under the acidic condition in the stomach, the HCT-AMPs transform to the helical conformation due to the protonation of the carboxylic groups, leading to the repression of the side-chain electrostatic interactions between the anionic carboxylate and cationic amine groups, ultimately leading to minimal toxicity to intestinal commensal bacteria, while under the acidic condition in the stomach, the HCT-AMPs transform to the helical conformation due to the protonation of the carboxylic groups, leading to the repression of the side-chain electrostatic interactions between the anionic carboxylate and cationic amine groups, ultimately leading to minimal toxicity to intestinal commensal bacteria.

### Results

**HCT-AMPs Display pH-Sensitive Helix-Coil Transition.** Random copolypeptides PL2. (PGA)\(_{m-r}^{-}(\text{PHLG-MHH})_{n}\), with anionic glutamic acid (Glu) and cationic poly(N-methylidexylanilinium)hexyl-l-glutamate (PHLG-MHH) residues (Fig. 1A) were developed via the ring-opening polymerization of \(L-\gamma-(6\text{chlorohexyl})\text{-Glu-based N-carboxyanhydrides (NCA)}\) and \(t\text{-urt-butyl-Glu-NCA (tBLG-NCA)}\) (i), followed by anination (ii) and trifluoroacetic acid-assisted desulfonation (iii) (44, 49) (SI Appendix, Scheme S2). pH-independent helical polypeptide and nonhelical polypeptides (prepared with D-L-\(\gamma-(6\text{chlorohexyl})\text{-Glu-NCAs}) and DL-\(t\text{-urt-butyl-Glu-NCAs}) as monomers) were synthesized as control polypeptides, named as P1L = (PHLG-MHH)\(_{25}\), PDL2 = (DFLG-MHH)\(_{25}\) (SI Appendix, Table S1 and Scheme S3). The structure of the polypeptides was confirmed by \(^1\text{H-NMR} \) spectra (SI Appendix, Figs. S1 and S2).

The secondary structure of the polypeptides at different pH value was then investigated by circular dichroism (CD). As shown in Fig. 1B, the secondary structure of PL2 was related to the charge status of PGA (pK\(_a\) \(\sim 4.5\)) (SI Appendix, Fig. S3). Particularly, at pH \(\geq 5.1\), when the carboxyl groups exhibited negative charge, PL2 adopted random coiled conformation due to the intermolecular electrostatic interaction between the negatively charged carboxyl groups and the positively charged amine groups of PHLG-MHH. In contrast, at pH \(\leq 4.2\) when the carboxyl groups were protonated, PL2 restored typical helical conformation as evidenced by the double minima at 208 and 222 nm, which was attributed to the depletion of side-chain charge interactions. Distortion of helical conformation was noted when the pH was adjusted back to neutral (Fig. 1C). In consistency with such findings, PL1 containing only the cationic PHLG-MHH segment displayed stable helical conformation independent of pH change (SI Appendix, Fig. S4A). PDL2, a racemic analog of PL2, also demonstrated pH-independent nonhelical conformation.

**HCT-AMPs Selectively Kill \textit{H. pylori} in vitro.** With respect to its pH-responsive secondary structure, the HCT-AMP is expected to show low toxicity at neutral pH while showing high antibacterial activity against \textit{H. pylori} in the acidic gastric environment. To test this hypothesis, we first evaluated the hemolytic activity of the polypeptides at pH 7.4. PL2 and PDL2, affording random coiled structure at pH 7.4, which showed no hemolytic activity at a high concentration up to 70 \(\mu\)M, while PL1 with stable helical structure caused remarkable hemolysis at 10 \(\mu\)M, which further substantiated the helical conformation-dependent membrane toxicity against erythrocytes (Fig. 2A). Additionally, PL2 showed low antibacterial activity against \textit{Escherichia coli} DH5\(\alpha\) and MG1655 at pH 7.4, affording the minimal inhibitory concentration (MIC) higher than 70 \(\mu\)M. Such results suggest that PL2 would not kill commensal bacteria in the intestine with relatively neutral pH. We then determined the bactericidal activity of HCT-AMPs against \textit{H. pylori} SS1 strain under various pH conditions. Upon incubation of SS1 with PL2 at pH 7.4 for 1 h, no bactericidal activity was noted. However, notable bacterial killing was achieved by PL2 at pH 4.0, and further decreased pH value decreased to higher bactericidal activity (Fig. 2B). As a comparison, the nonhelical PDL2 exhibited unappreciable bactericidal activity at both pH 7.4 and 3.0 and the helical PL1 killed \textit{H. pylori} SS1 at both conditions (SI Appendix, Fig. S4 B and C). These results collectively indicate that the restoration of helical structure is essential for PL2 to selectively kill \textit{H. pylori} under the gastric acidic condition, and the loss of helical conformation under intestinal neutral condition may contribute to the low toxicity against commensal bacteria.

A combination of omeprazole, amoxicillin, and clarithromycin (OAC) is used for the treatment of \textit{H. pylori} infection in clinic (8). We further explored the bactericidal activity of these antibiotics under the same condition to allow direct comparison with PL2. Although the MIC of amoxicillin and clarithromycin against SS1 was as low as 0.13 and 0.07 \(\mu\)M, respectively, amoxicillin and clarithromycin only killed \(\sim40\%\) and \(\sim60\%\) of the bacteria at pH 7.4 after 1 h incubation at high concentrations of 273.7 and 133.7 \(\mu\)M, respectively, indicating the slow function of antibiotics (SI Appendix, Fig. S5A). Moreover, these two antibiotics showed no bactericidal activity against SS1 at pH 3.0, rationalizing why PPI is demanded to raise the pH of the stomach to potentiate the antibiotic-mediated treatment of \textit{H. pylori} infection in vivo. A combination of OAC effectively killed SS1 in 1 h at both pH 7.4 and 3.0 (SI Appendix, Fig. S5B). The antibacterial activity of OAC at pH 3.0 was attributed to the bacterial killing effect of omeprazole, while a combination of amoxicillin and clarithromycin (AC) showed no antibacterial activity at pH 3.0 (SI Appendix, Fig. S5C). It should
HCT-AMPs effectively kill clinically isolated drug-resistant \( \mu \) under acidic condition broth (BB) medium supplied with fresh urea. HCT-AMPs selectively kill \( E \) Brucella which well correlated to the helical and nonhelical conformation of PDL2 induced unappreciable dye leakage under both conditions, PL1 induced notable dye leakage at both pH 7.4 and 4.0, while PDL2 did not affect the morphology of bacteria under both conditions (SI Appendix, Fig. S4E). Taken together, these results indicate that HCT-AMPs are able to selectively kill bacteria under acidic condition via acid-triggered helix formation and helix-assisted bacterial membrane disruption.

The emergence of drug-resistant \( H. \) pylori is the main reason for clinical treatment failure (29, 30, 33). The resistance to clarithromycin, metronidazole, tetracycline, fluoroquinolones, as well as rifamycin, has become a serious issue (29, 30, 33). As such, we further tested the antibacterial activity of HCT-AMPs against clinically isolated strains, including clarithromycin-resistant J99A-7, J99A-11, J99C-8, and J99D-1 (51, 52) (SI Appendix, Fig. S5A and D). PL2 effectively killed over 90% of these bacterial strains at pH 3.0 after 1 h incubation at a concentration of 4.4 \( \mu \)M (Fig. 3), which further substantiated its potency in overcoming the drug resistance toward effective anti-\( H. \) pylori therapy. OAC could not effectively kill drug-resistant bacteria J99A-11 and J99D-1 at pH 7.4. It instead killed bacteria at pH 3.0 in a concentration-dependent manner (Fig. 3B and SI Appendix, Fig. S5F), which was also attributed to the bacterial killing effect of omeprazole (Fig. 3C and SI Appendix, Fig. S5G).

The application of AMPs often hurdles by the short durations of antimicrobial activity due to their rapid digestion by endogenous proteases (37). The RA polypeptide, with densely packed hydrophobic side chains forming a hydrophobic cortex to protect the polypeptide backbone amide bonds, was shown to be more be noted that omeprazole performs differently in vitro and in vivo. Omeprazole is used for the increase of the gastric pH to enhance the antimicrobial activity and stability of antibiotics in the gastric fluid in vivo (5–8). It suppresses stomach acid secretion via specific inhibition of the \( H^+/K^-\)ATPase system found at the secretory surface of gastric parietal cells. Because of such unique mechanism, omeprazole cannot increase the pH value of the acidic medium during the bacterial killing study in vitro. Instead, omeprazole readily converts to the active sulfenamide form and causes a substantial decrease in survival of \( H. \) pylori under acidic condition in vitro (50).

The bactericidal mechanism of HCT-AMPs was further explored by the vesicle leakage assessment as well as bacterial morphology observation. PL2 was labeled with Cy5 (Cy5-PL2), and its binding with bacteria membranes was observed using fluorescence microscopy. More Cy5-PL2 bound to SS1 bacterial cells at pH 4.0 than at pH 7.4 (SI Appendix, Fig. S6), which was attributed to the formation of cationically helical structure at acidic pH that promoted binding of polypeptides to phospholipid bilayers. The membrane activity of the polypeptides was studied at both pH 7.4 and 4.0 by assessing dye leakage from anionic liposomes, a commonly used model to simulate the phosphatidylethanolamine-rich bacterial cell membrane (44). After incubation, PL2 induced great dye leakage from the liposome at pH 4.0 while inducing minimal dye leakage at pH 7.4 (Fig. 2C). These results demonstrate that the acid-triggered helix formation of HCT-AMPs allows them to directly disrupt the bacterial membranes, a mechanism that most AMPs utilize to kill bacteria. Comparatively, control polypeptide PL1 induced notable dye leakage at both pH 7.4 and 4.0, while PDL2 induced unappreciable dye leakage under both conditions, which well correlated to the helical and nonhelical conformation of PL1 and PDL2, respectively (SI Appendix, Fig. S4D). In support of the acid-activated membrane disruption, we further observed dramatic damage of the bacterial membranes by scanning electron microscopy (SEM) after incubation with PL2 at pH 4.0 (Fig. 2D). At pH 7.4, PL2 minimally affected the bacterial morphology, consistent with its minimal membrane activity on the nonhelical state. Control polypeptide PL1 showed membrane disruption toward SS1 at both pH 7.4 and 4.0, while PDL2 did not affect the morphology of bacteria under both conditions (SI Appendix, Fig. S4E).

**Fig. 2.** HCT-AMPs selectively kill \( H. \) pylori under acidic condition in vitro. (A) The hemolytic activity of polypeptides at pH 7.4. PL1, PL2, and PDL2, dissolved in PBS (pH 7.4) at various concentrations, was incubated with fresh rabbit blood for 1 h. Hemoglobin release was measured by UV absorbance at 576 nm using a microplate reader. (B) The survival rate of \( H. \) pylori SS1 after incubation with PL2 for 1 h at various pHs. PL2, dissolved in the Tris-HCl buffer at various pHs (pH 7.4, 4.0, 3.0), was incubated with SS1 at corresponding pHs in Brucella broth (BB) medium supplied with fresh urea (10 mM), 10% FBS, and vancomycin (5 \( \mu \)g/ml). The bacterial count was determined by counting colony-forming units (cfu) of alive bacteria with agar plating. Bacteria incubated with Tris HCl buffer only at corresponding pH were served as 100% survival. (C) Extent of ANTS/DPX efflux in negatively charged liposomes after treatment with PL2 at various concentrations at pH 7.4 or 4.0. (D) SEM images of SS1 after treatment with Tris-HCl buffer. Instead, omeprazole performs differently in vitro and in vivo. Omeprazole is used for the increase of the gastric pH to enhance the antimicrobial activity and stability of antibiotics in the gastric fluid in vivo (5–8). It suppresses stomach acid secretion via specific inhibition of the \( H^+/K^-\)ATPase system found at the secretory surface of gastric parietal cells. Because of such unique mechanism, omeprazole cannot increase the pH value of the acidic medium during the bacterial killing study in vitro. Instead, omeprazole readily converts to the active sulfenamide form and causes a substantial decrease in survival of \( H. \) pylori under acidic condition in vitro (50).

The bactericidal mechanism of HCT-AMPs was further explored by the vesicle leakage assessment as well as bacterial morphology observation. PL2 was labeled with Cy5 (Cy5-PL2), and its binding with bacteria membranes was observed using fluorescence microscopy. More Cy5-PL2 bound to SS1 bacterial cells at pH 4.0 than at pH 7.4 (SI Appendix, Fig. S6), which was attributed to the formation of cationically helical structure at acidic pH that promoted binding of polypeptides to phospholipid bilayers. The membrane activity of the polypeptides was studied at both pH 7.4 and 4.0 by assessing dye leakage from anionic liposomes, a commonly used model to simulate the phosphatidylethanolamine-rich bacterial cell membrane (44). After incubation, PL2 induced great dye leakage from the liposome at pH 4.0 while inducing minimal dye leakage at pH 7.4 (Fig. 2C). These results demonstrate that the acid-triggered helix formation of HCT-AMPs allows them to directly disrupt the bacterial membranes, a mechanism that most AMPs utilize to kill bacteria. Comparatively, control polypeptide PL1 induced notable dye leakage at both pH 7.4 and 4.0, while PDL2 induced unappreciable dye leakage under both conditions, which well correlated to the helical and nonhelical conformation of PL1 and PDL2, respectively (SI Appendix, Fig. S4D). In support of the acid-activated membrane disruption, we further observed dramatic damage of the bacterial membranes by scanning electron microscopy (SEM) after incubation with PL2 at pH 4.0 (Fig. 2D). At pH 7.4, PL2 minimally affected the bacterial morphology, consistent with its minimal membrane activity on the nonhelical state. Control polypeptide PL1 showed membrane disruption toward SS1 at both pH 7.4 and 4.0, while PDL2 did not affect the morphology of bacteria under both conditions (SI Appendix, Fig. S4E). Taken together, these results indicate that HCT-AMPs are able to selectively kill bacteria under acidic condition via acid-triggered helix formation and helix-assisted bacterial membrane disruption.
stable against proteolysis compared with typical AMPs (44). We herein also tested the proteolytic stability of HCT-AMPs against pepsin, the main digestive protease in the stomach. After incubation with pepsin at pH 4.0 for up to 24 h, HPLC analysis showed that PL2 was resistant to pepsin-mediated degradation (SI Appendix, Fig. S7).

**HCT-AMPS Selectively Kill *H. pylori* in Vivo.** We then evaluated the therapeutic efficacy of HCT-AMPs against *H. pylori* SS1 in vivo. We first studied the biodistribution of HCT-AMPS after oral gavage of Cy5-labeled polypeptides. PL2 and PDL2 showed similar biodistribution profiles, with the majority of the polypeptides retained in the stomach and intestines within 4 h and gradually excreted within 24 h (Fig. 4A). By collecting major organs 4 h postoral gavage, we further observed strong fluorescence in the gastric tissue (Fig. 4 B and C). Such observation was further supported by the quantification of the gastric retention of PL2, which reached ∼40% ID/g at 4 h postgavage and notably decreased to ∼6% 24 h postgavage (Fig. 4D). It should be mentioned that 4 h is a relatively long time compared with the reported gastric emptying time of mice (3). As such, these results indicate that HCT-AMPS could be effectively retained in the stomach against gastric emptying, likely due to the electrostatic interactions between the anionic mucus and the polypeptides that possess positive charges under acidic condition. We further studied the penetration of Cy5-PL2 into gastric mucosa by observing the cryosections of mouse stomach collected 4 h after oral gavage. The confocal image revealed a thin layer of Cy5-PL2 on the luminal side of the gastric mucosa, confirming the diffusion of PL2 toward the gastric epithelium and its retention in the mucus layer (Fig. 4E).

We then infected mice with *H. pylori* SS1 (1 × 10^8 cfu per animal) by oral gavage every other day for four times (Fig. 5A). Two weeks after inoculation, infected mice were divided into four groups and treated with PBS (control), triple-therapy OAC, PDL2, or PL2. PL2 showed similar bacteria killing ability as the OAC treatment, with a decrease of bacterial burden by ∼100× compared with the control group. PDL2, although displaying similar biodistribution profiles as PL2, showed no significant decrease of bacterial burden (Fig. 5B). These results indicate that the formation of membrane-active helical structure is essential for killing *H. pylori*. Because the pH in the mouse stomach (∼3.0 when fasted and ∼4.0 when fasted) is higher than that in the human stomach (53), it is expected that the HCT-AMPS may show even higher *H. pylori* killing efficiency in the human stomach.

The toxicity of PL2 was further explored. No obvious change of animal body weight was noticed following treatment with PL2 as described above, indicating the low toxicity of PL2 in vivo (Fig. 5C). To analyze the toxicity of PL2 toward the stomach, we performed H&E-staining assay, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, and detected caspase 3/8 activity of mouse stomach after treatments. No significant inflammation of stomach or injury of mucosa layer was observed after gavage of PL2 according to the H&E-staining assay (SI Appendix, Fig. S8 A–C). PL2 administration did not induce apoptosis in stomach cells, as measured by the TUNEL assay and the activities of caspase 3 and 8 (SI Appendix, Fig. S8 D–F).
D–G). The plasma levels of alanine aminotransferase, aspartate aminotransferase, creatinine, urea nitrogen, sodium ion, and potassium ion showed no significant change after the PL2 treatment (SI Appendix, Table S2), verifying the lack of significant acute damage toward the liver and kidney as well as the balance of electrolytes in the blood. The low toxicity of PL2 was further confirmed by H&E staining of intestines, liver, and kidney, with barely detectable histological abnormality (SI Appendix, Fig. S9). More importantly, PL2 did not cause undesired killing of the commensal bacteria in the ileal contents and feces of mice, while OAC treatment killed commensal bacteria in the ileal contents and feces by 65% and 86%, respectively (Fig. S5D). These results collectively indicate the low side effect of HCT-AMPs toward normal cells or commensal bacteria.

**Discussion**

In this study, we designed a class of pH-responsive HCT-AMPs as a single bactericidal agent that can specifically target *H. pylori* under the acid condition in the stomach. Random polypeptide copolymers ((PGA)ₙ-t-(PHLG-MHH)ₙ) were developed with randomly distributed negatively charged Glu residues and positively charged PHLG-MHH residues. The conformation and membrane activity of the polypeptides depend on the charge state of Glu residues. At physiological pH, the polypeptides adopt random coiled conformation with low toxicity, while in the stomach under acidic condition they are converted to the helical conformation with potent membrane disruption capability to effectively kill *H. pylori*. With such design, HCT-AMPs showed minimal toxicity against normal tissues/commensal bacteria and showed minimal toxicity against normal tissues/commensal bacteria but in vivo *H. pylori* killing efficacy comparable to the triple therapy, with remarkably improved selectivity of anti-*H. pylori* therapy.

Commensal bacteria have gained increased attention due to their important functions during the physiological and metabolic processes as well as the development of the immune system (11, 13, 14). Undesired alteration of the microbiome can disturb the symbiotic relationship between resident microorganisms and the digestive tract, and thus induce the occurrence as well as progression of various diseases, including but not limited to inflammatory bowel disease (16, 17), colon cancer (18, 19), Parkinson’s disease (20), obesity (21–23), diabetes (24), atherosclerosis (25, 26), and allergy (27, 28). Recent research has also shown that the elimination of commensal bacteria significantly alleviates the efficacy of anticancer therapeutics, including CTLA-4 and PD-L1 blockade-mediated cancer immunotherapy (54–56). Therefore, development of therapeutics that can selectively kill *H. pylori* without harming commensal bacteria is highly attractive and important. The triple therapy, used in clinical *H. pylori* treatment, killed 65% and 86% of the commensal bacteria in the ileal contents and feces, respectively, while the pH-sensitive HCT-AMPs showed unappreciable toxicity against commensal bacteria (Fig. 5D). This strategy therefore represents an ideal and promising approach to target and selectively kill *H. pylori* in the stomach without provoking damage to commensal bacteria.

Apart from the undesired toxicity, the triple therapy also suffers from the progressive increase of drug resistance that undermines its therapeutic efficacy against *H. pylori*-induced gastric diseases (29–31, 33, 57). High resistance rates are noted for clinically used antibiotics, such as 60–70% for metronidazole, 20–38% for clarithromycin, and 30–38% for levofloxacin (57). In the current study, HCT-AMPs kill bacteria mainly by disrupting the membrane integrity (Fig. 2D), a highly destructive mechanism for bacterial killing with low susceptibility for resistance development (34–37, 58, 59). As such, we demonstrated that HCT-AMPs could effectively kill clinically isolated drug-resistant bacterial strains (Fig. 3A). The clinical success of triple therapy also faces great hurdles by the resistance to PPI, because in many patients, PPI cannot effectively increase their gastric pH, thus leading to low antimicrobial activity of antibiotics in the gastric fluid (32, 33). The HCT-AMPs developed herein kill *H. pylori* under acidic condition as a single agent, and they exhibited increased anti-*H. pylori* efficacy with the decrease of pH (Fig. 2B), effectively bypassing the problem of PPI resistance. Based on these understandings, it is expected that the HCT-AMPs would outperform the classical triple therapy toward anti-*H. pylori* therapy.

In conclusion, we developed a class of pH-sensitive HCT-AMPs as a single therapeutic agent to target and selectively treat *H. pylori* infection in the stomach. The HCT-AMPs can specifically kill *H. pylori*, including drug-resistant strains, under acidic conditions, and showed minimal toxicity against normal tissues/commensal bacteria. The pH-sensitive HCT-AMPs greatly outperform the triple therapy that suffers from undesired toxicity as well as drug resistance. This strategy therefore provides a safe and effective approach to overcome the critical challenges in the treatment of...
**Materials and Methods**

**Animals.** Female C57BL/6J mice were purchased from The Jackson Labs (Bar Harbor, ME) for the efficacy studies and biodistribution studies. Feed and water were available ad Librium. Artificial light was provided in a 12 h:12 h cycle. The animal protocol was reviewed and approved by the Illinois Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Urbana–Champaign. For the toxicity studies, male ICR mice (6–8 weeks, 18–22 g) were obtained from Shanghai Slasco Experimental Animal Co., Ltd (Shanghai, China) and were housed in a specific pathogen-free room. The animal protocols were approved by the Institutional Animal Care and Use Committee, Soochow University.


**Cheng J.** Acknowledges partial support from the NSF 15430145, 15173123, and 15172205, the Ministry of Science and Technology of China (2016YFA0201200), and Priority Academic Program Development of Jiangsu Higher Education Institutions. R.M.P. acknowledges support from the NIH (RO1 DK 58587, RO1 CA 77955, PO1 CA 116087, and P30 DK 059040).

**PL2** was synthesized via the reaction of PCHLG2F-PtBLG18 with N-methyldiethylamine followed by removal of the tert-butyl group by trifluoroacetic acid. Details describing the formulation and characterization of HCT-AMPs, in vitro and in vivo antibacterial assays, and hemolytic assay can be found in SI Appendix.

**Acknowledgments.** J. Cheng acknowledges partial support from the NSF (CHE 15087110 and 130848) for the design and synthesis of polypeptides. L.-F.C. acknowledges support from the NIH (R21AI117080) for antibacterial peptides against H. pylori. L.Y. acknowledges support from the National Natural Science Foundation of China (51403145, 51573123, and 51722205), the Ministry of Science and Technology of China (2016YFA0201200), and Priority Academic Program Development of Jiangsu Higher Education Institutions. R.M.P. acknowledges support from the NIH (R01 DK 58587, R01 CA 77955, P01 CA 116087, and P30 DK 059040).

**References**

9. Cheng J. Acknowledges partial support from the NSF 15430145, 15173123, and 15172205, the Ministry of Science and Technology of China (2016YFA0201200), and Priority Academic Program Development of Jiangsu Higher Education Institutions. R.M.P. acknowledges support from the NIH (R01 DK 58587, R01 CA 77955, P01 CA 116087, and P30 DK 059040).