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The Pexiganan-Loaded Chitosan Nanoparticle: Preparation, Characterization and Antibacterial Activity on *Helicobacter pylori*

Abstract

Helicobacter pylori is Gram-negative, spiral shape, as well as microaerophilic pathogen that be able to establish chronic gastrointestinal infection. H. pylori is linked with severe digestive disease i.e. peptic ulceration, as well as gastric adenocarcinoma. Herein, we designed and characterized sustained release nanoparticles of chitosan-pexiganan for possible elimination of H. pylori infection in mice model.

Keywords: *Helicobacter pylori*; Pexiganan; Antimicrobial peptids; Chitosan; Cure rate

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Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative, spiral shape, as well as microaerophilic bacteria that frequently infects children, particularly in developing countries. This bacterium is considered one of the most popular infectious diseases which colonized in gastric mucosa of nearly 4.4 billion individuals worldwide (1-2). *H. pylori* can establish a successful chronic infection that be associated with sever digestive diseases e.g. chronic atrophy gastritis, peptic ulcer, as well as gastric adenocarcinoma (3-5). According to the Maastricht II 2000 consensus statement, the first-line eradication therapy for treatment of *H. pylori* infection is include combination of several concomitant antibiotics plus a proton pump inhibitors (PPIs); However, it is obviously that antibiotic resistance that be consider as the main cause of declining *H. pylori* cure rate below 80% (6-8). In addition, short residence time of antimicrobial agents in the human stomach, treatment costs, CYP2C19 polymorphism, adverse outcomes, and poor compliance lead to decline of *H. pylori* cure rate (9-10). Antimicrobial peptides (AMPs) is natural short peptides that have broad spectrum antimicrobial activity without significant toxicity against human cell lines; Pexiganan is a 22-amino-acid peptide with superior anti-*H. pylori* activity in previous in vitro studies (11-12). Nevertheless, the local treatment of *H. pylori* by oral administration route may not be sufficient to penetrate to the human stomach's baseline (13). limited bioavailability of the antibacterial agents is important factor hat warranted *H. pylori* eradication (14-16). Delivery of drugs efficiently into the body or fundus of the stomach could be helpful strategy to achievement of efficacious *H. pylori* cure with further recurrence (17-18). Chitosan is a natural polysaccharide that naturally biodegradable, nontoxic, polycationic, biocompatible (19). Chitosan nanoparticles (CsNPs) is a water soluble formulation with sustained release and mucous-adhesive structures that disrupted tight junction that simplified entrance of hydrophilic macromolecules e.g. antimicrobial peptides to sub-mucosal tissue by depletion of the mucosal barriers (20-22). This study was preliminary report of a chitosan-loaded pexignan nanoparticles delivery system against *H. pylori* infection.

Materials and methods

Cloning, expression, and purification of pexiganan were done as previously described (23). Chitosan nanoparticles (CsNPs) were prepared using the ionic elation technique according to Morles et al., 2021 (24). Briefly, we previously optimized Chitosan: TPP (sodium tripolyphosphate) nanoparticles in a different ratio. An aqueous solution of 9 ml of sodium tripolyphosphate (TPP) (2 mg/ml, pH 5) was added drop-wise to 17.5 ml in chitosan solution (1 mg/ml) containing pexignan

that stirred for overnight. After overnight stirring, the pexiganan-loaded chitosan nanoparticles (PLCsNPs) were harvested via centrifugation at 8000 rpm at 4 °C for 30 min. Subsequently, the PLCsNPs were investigated for further characterizations such as particle size and polydispersity index, scanning electron microscopy, X-ray diffraction (XRD), entrapment and loading efficiencies, in vitro release studies, in vitro activity against clinical *H. pylori* isolates, as well as in vivo *H. pylori* eradication analysis (25).

Results and Discussion

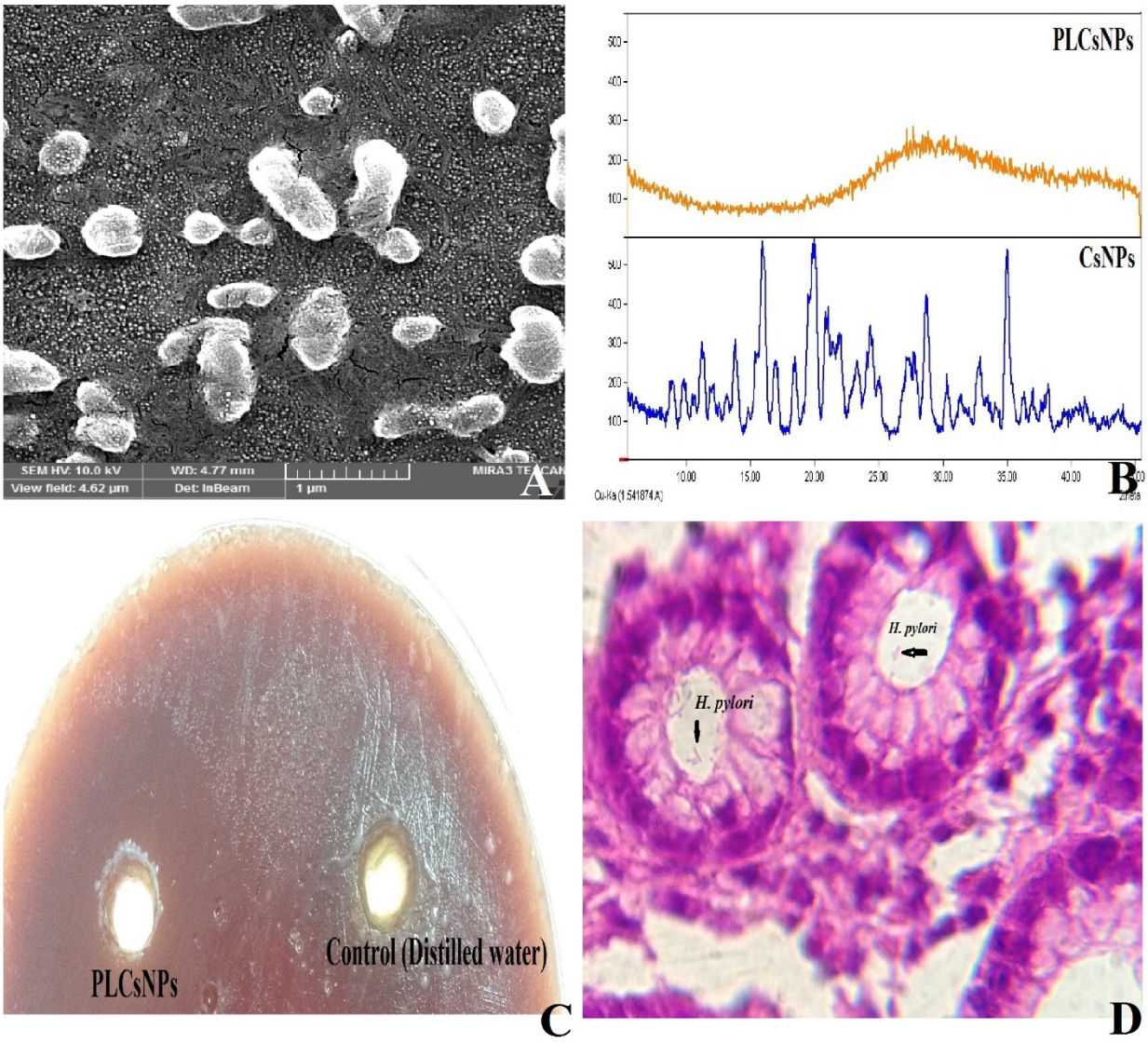
We prepared Pexiganan-loaded chitosan nanoparticles (PLCsNPs) to reach solubility, stability, and bioavailability of pexigana against *H. pylori* infection. To our knowledge, this is the first effort to synthesize PLCsNPs. CsNPs and PLCsNPs have an average mean diameter of about 183 ± 21.2 nm and 203 ± 12.6 nm, respectively. According to dynamic light scattering (DLS), the zeta potential was also -12.6 ± 3.1 mV and -17.3 ± 8.5 mV, respectively. According to the literatures, the particle size and surface charge indexes confirmed the stability of PLCsNPs (26-27).

SEM results also suggested that PLCsNPs have separate, round shape as well as condensed construction (Fig. 1a). In addition, the entrapment and loading efficiencies of pexiganan loaded nanoparticles system was found to be approximately 46.6% and 35.7%, respectively.

The assessment of in vitro sustained-release of pexiganan was accomplished by the direct dispersion method at pH dependent with different pH 3, 5, and 7.4. After spanning a period up to of 120 hours, approximately 80% of the drug was observed to be released by this time. During XRD analysis, we did not find any characteristic diffraction peak that represents a modification of the diffraction peak caused to the physicochemical amendment. This result was confirmed by previous relevant studies (28-29). The crystalline phase may be turned to an amorphous shape (Fig. 1b). As shown in Fig. 1c, *H. pylori* clinical strains were susceptible to pexiganan as well as pexiganan-loaded chitosan nanoparticles (PLCsNPs).

The Agar dilution test revealed that the MIC of pexiganan and PLCsNPs for *H. pylori* strains is 4-8 µg/mL previously investigated by Zhang et al., 2015 (30). Specific pathogen-free female C57BL/6 mice aged 6-8 weeks' old were obtained from Mashhad University of Medical Sciences. Mice were inoculated intragastrically with suspensions containing 10+9 CFU *H. pylori* clinical strains, five times in 10 days. Pexiganan and PLCsNPs were orally administered at doses of 2, 5, and 10 mg/kg once every day for seven consecutive days. As shown in Fig. 1d, *H. pylori*-infected mouse stomachs during the animal challenge. After the treatment course, the results of the urease test showed that there are significant differences between the pexiganan and PLCsNPs groups. Our findings suggested that the *H. pylori* eradication was higher in mice received with PLCsNPs than pexiganan (p-value < 0.01).

Figure 1: Characteristics of pexiganan-loaded chitosan nanoparticles (PLCsNPs).



The bacterial colonization density was listed in Table 1 evaluated by urease activities at OD: 550 nm, the group receiving PBS as a negative control harboring a high count of bacteria about 107 (CFU/stomach). There is an inverse association between the average bacterial count and orally administered pexiganan dosage; However, a complete *H. pylori* cure was not obtained even at a high peptide dose. CsNPs have a great role in the specific delivery of the pexignan to the mice's gastric mucosa. Surprisingly, mice under *H. pylori* treatment with PLCsNPs were completely cleared at a dosage of 5 and 10 mg/kg similar to the mice group that received clarithromycin (Table 1). Consistently, Zhang et al., 2015 also confirmed the efficacy of pexiganan nanoparticles (PNPs) in the treatment of *H. pylori* infection in Kunming mice and Sprague-Dawley rats (30).

Table 1: the effect of pexiganan and pexiganan-loaded chitosan nanoparticles against *H. pylori* infection in C57BL/6 mice

Drug type	**Dosage (mg/kg)**	***H. pylori* cure rate (%)**	**Colonization density (Log CFU/Stomach)**
Water	0	0/6 (0)	7.25 ± 0.3
Pexiganan	2	0/6 (0)	7.58 ± 0.1
Pexiganan	5	1/6 (17)	3.42 ± 0.6
Pexiganan	10	3/6 (50)	2.13 ± 0.9
CsNPs	0	0/6 (0)	7.18 ± 0.5
PLCsNPs	2	3/6 (50)	2.05 ± 0.2
PLCsNPs	5	6/6 (100)	NA

PLCsNPs	10	6/6 (100)	NA
Clarithromycin	20	6/6 (100)	NA

Conclusion

In summary, conventional administration of pexiganan has insufficient eradication of *H. pylori* infection due to the short duration of contact with gastric mucosa and low bioavailability. Nevertheless, the encapsulation of pexiganan within the chitosan-based nanoparticles enhanced pexiganan-related anti-*H. pylori* activity by improvement of water solubility, site-specific, bioadhesive, sustained release, as well as bioavailability of pexiganan. Thus, PLCsNPs brought a new avenue to the development of novel therapeutic agents as potential candidates for *H. pylori* cure for efficient prevention of further gastric cancer.

Conflict of interest

There is no conflict of interest.

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Ethical statement

All of the experiments were ethically approved by the Institutional Ethical Committee and Research Advisory Committee of Mashhad University of Medical Sciences and carried out according to their recommendations under registration number (IR.MUMS.MEDICAL.REC.1400.144). The study was also performed in compliance with the ARRIVE guidelines.

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