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Anti–*Helicobacter Pylori* Effect of Mucoadhesive Nanoparticles Bearing Amoxicillin in Experimental Gerbils Model

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ABSTRACT

The purpose of the present study was to design mucoadhesive gliadin nanoparticles (GNP) containing amoxicillin and to evaluate their effectiveness in eradicating *Helicobacter pylori*. GNP-bearing amoxicillin (AGNP) was prepared by desolvation method. The effect of process variables such as gliadin concentration and initial drug loading on particle size, shape, percent payload, percent entrapment efficiency, in vitro release profile, and mucoadhesive property of GNP was assessed. Rhodamine isothiocyanate–entrapped GNP formulations were prepared to evaluate their in vivo gastric mucoadhesive property in albino rats. With increasing gliadin concentration, the mucoadhesive property of GNP increased. Typically, the maximum amount of nanoparticles remaining was 82 ± 4%, which represented a stronger mucoadhesive propensity and specificity of GNP toward the stomach. In vitro antimicrobial activity of AGNP was evaluated by growth inhibition studies on an isolated *H pylori* strain. The time required for complete eradication was higher in AGNP than in amoxicillin because of the controlled drug delivery of amoxicillin from AGNP. In vivo clearance of *H pylori* following oral administration of AGNP to infected Mongolian gerbils was examined. Amoxicillin and AGNP both showed anti–*H pylori* effects in this experimental model of infection, but the required dose for complete eradication was less in AGNP than in amoxicillin. In conclusion, AGNP eradicated *H pylori* from the gastrointestinal tract more effectively than amoxicillin because of the prolonged gastrointestinal residence time attributed to mucoadhesion. A dosage form containing mucoadhesive nanoparticles bearing a potential antibiotic should be useful for the complete eradication of *H pylori*.

KEYWORDS: gliadin, mucoadhesion, nanoparticles, *H pylori*, amoxicillin.

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INTRODUCTION

In a relatively short time span, *Helicobacter pylori* has become recognized as a major gastric pathogen with worldwide distribution. *H pylori*, a prevalent human-specific pathogen, is a causative agent in chronic active gastritis,[1] gastric and duodenal ulcers,[2] and gastric adenocarcinoma,[3] one of the most common forms of cancer in humans. *H pylori* is susceptible to many antibiotics in vitro but has proved difficult to eradicate in vivo.

Recent biopsy studies[4,5] and cell culture infection models[6-8] have provided increasing evidence for the intracellular localization of *H pylori*. Once acquired, *H pylori* penetrates the gastric mucus layer and fixes itself to various phospholipids and glycolipids in the mucus gel. Therefore, access of antimicrobial drugs to the site is restricted from both the lumen of the stomach and the gastric blood supply. *H pylori* may also have acquired resistance to the commonly used antimicrobial agents. Because conventional drug delivery systems do not remain in the stomach for prolonged periods, they are unable to deliver the antibiotics to the site of infection in effective concentrations and in fully active forms. Also, many antimicrobial agents, such as penicillin and erythromycin, degrade rapidly in an acidic environment.

It is therefore necessary to design drug delivery systems that cannot only alleviate the shortcomings of conventional delivery vehicles but also deliver the antimicrobials to the infected cell lines. The absorption of an antibiotic into the mucus through the mucus layer (from the gastric lumen) is believed to be more effective for *H pylori* eradication than absorption through the basolateral membrane (from blood). A preparation that spreads out, adheres to the gastric mucosal surface, and continuously releases antibiotic should be highly effective against *H pylori*. Various mucoadhesive drug delivery systems have been proposed in *H pylori* eradication, such as carboxyvinyl mucoadhesive microspheres,[9] chitosan microspheres,[10] Eudragit floating microspheres,[11] and cholestyramine microcapsules.[12]

The use of nanoparticles is of interest for bioadhesion purposes because these pharmaceutical dosage forms have a large specific surface, which is indicative of a high interactive potential with biological surfaces. Gliadin appears to be a suitable polymer for the preparation of mucoadhesive

nanoparticles capable of adhering to the mucus layer. It has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Its hydrophobicity and solubility permit the design of nanoparticles capable of protecting the loaded drug and controlling its release.[13] Gliadin nanoparticles (GNP) have shown a great tropism for the upper gastrointestinal regions, and their presence in other intestinal regions has been shown to be very low.[14]

Materials and Methods

Materials

Gliadin, trifluoroacetic acid (TFA), and rhodamine isothiocyanate (RITC) were purchased from Sigma (St. Louis, MO). Amoxicillin trihydrate was purchased from Zoetic Formulations Ltd (Chennai, India). Pluronic F 68, modified Skirrow's medium, Brucella broth, and fetal calf serum (FCS) were purchased from Himedia (Mumbai, India). All other chemicals used were of reagent grade.

Preparation of GNP

GNP were prepared by a desolvation procedure described previously.[15] Briefly, various amounts of gliadin and amoxicillin were dissolved in 20 mL of an ethanol:water phase (7:3 vol/vol), and this solution was poured into 40 mL of a stirred physiological saline phase (0.9% wt/vol of NaCl in water) containing 0.5% Pluronic F-68 as a stabilizer. Ethanol was removed by evaporation under reduced pressure (Buchi RE-140, Buchi Labortechnik AG, Switzerland). GNP prepared in this way were purified by centrifuging twice (Eltech centrifuge, SICO, Mumbai, India) at 20 000 rpm for 15 minutes. The supernatants were removed and the pellets were washed with distilled water 3 times. Then they were resuspended in phosphate buffered saline (PBS; pH 7.4, ionic strength 0.15M). Finally, the nanoparticles were freeze-dried using 5% glucose solution as a cryoprotector.

Particle Size and Morphology

The particle size, size distribution, and zeta potential of GNP were determined in a Zetasizer 3000 HS (Malvern Instrument Ltd, Worcestershire, UK). The surface morphology and internal structure of GNP were determined by scanning electron microscopy (SEM, Jeol JX 840-A, Tokyo, Japan). The samples were scanned, and photographs were taken (Figure 1). After the GNP suspension was digested, the nanoparticle yield was determined by spectrophotometry on a Shimadzu UV/Visible 1601 spectrophotometer (Tokyo, Japan) at 230.6 nm.[13]

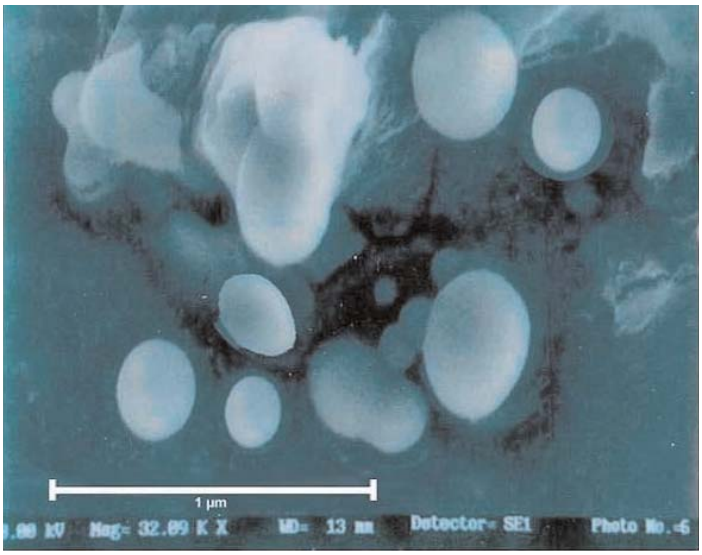

Figure 1. Scanning electron microphotograph of GNP: gliadin 50 mg and amoxicillin 80 mg.

Drug Entrapment Study

An appropriate amount of freeze-dried GNP was digested with a minimum amount of aqueous ethanolic solution. The digested homogenates were centrifuged at 15 000 rpm for 30 minutes, and supernatant was analyzed for drug content (C_1). Before digestion, the nonencapsulated drug remaining in the supernatant after the first centrifugation (C_2) was also estimated. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was followed for estimation of amoxicillin as described previously.[16] The instrument (Shimadzu) used consisted of a Shimadzu pump LC-10AT VP equipped with universal injector 77251 (Rheodyne) with an injection volume of 20 µL; an SPD-10A VP variable wavelength UV-Vis detector (Shimadzu); and Shimadzu Class-VP software version 5.03. A C-18 reverse-phase column (Luna; particle size 5 µL, column size 250 × 4 mm) was used. A mixture of trifluoroacetic acid 0.01M/CH_3OH (80:20) at the flow rate of 1 mL/min was used as the mobile phase, and detection was performed at 270 nm. Amoxicillin was quantified by using a calibration curve (concentration vs peak area) in a concentration range of 2 to 20 µg/mL with a correlation coefficient of 0.9942. By using C_1 and C_2, we calculated percent payload (Equation 1) and percent drug entrapment efficiency (Equation 2) were calculated as follows:

$$\% \ Payload = \frac{(C_1)}{GNP \ yield} \times 100 \qquad (1)$$

$$\% \ Entrapment \ efficiency = \frac{C_1}{(C_1 + C_2)} \times 100 \qquad (2)$$

Drug Release Studies

The drug release from nanoparticles was determined by suspending a dialysis tube containing the known quantity (50 mg) of the nanoparticles in a water-jacketed beaker containing 100 mL of simulated gastric fluid (SGF, pH 1.2) at 37 ± 1°C. The contents of the beaker were agitated on a magnetic stirrer. Samples were withdrawn periodically and replaced with an equal volume of fresh SGF (pH 1.2). Samples were diluted suitably and filtered through a filter paper (0.22 μm). Two different dissolution fluids, SGF (pH 1.2) with pepsin and SGF (pH 1.2) without pepsin, were used to study the effect of pepsin on the release rate of amoxicillin from GNP. The amoxicillin content was determined by HPLC.

Stability Studies of Gliadin and Amoxicillin

Ten milligrams of gliadin formulations was incubated at 37 ± 1°C with 20 mL of SGF (pH 1.2) for 24 hours and the suspension was centrifuged at 15 000 rpm for 1 hour, the supernatant was removed, and the nanoparticles were dissolved in ethanolic solution (7:3 ratio). The stability of gliadin solution was investigated by RP-HPLC method.[17] The experiments were performed as follows: instrument, Beckman (solvent molecule 126, software System Gold); column, Nucleosil C_{18} (5 μm, 30 nm), 4.6 × 240 nm; temperature, 50°C; sample volume, 100 μL; and elution system, linear gradient of acetonitrile and water, each containing 0.2% TFA. In addition, the mobile phase was filtered through a 0.45-μm pore-size membrane filter at a flow rate of 1 mL/min. The absorption wavelength of the detector was set at 220 nm. The percentage of the gliadin remaining was calculated by dividing the peak area of the gliadin at time 24h against the peak area of the gliadin at time zero multiplied by 100%. The degradation of gliadin was assumed to follow pseudo–first-order kinetics. The half-life ($t_{1/2}$) and the shelf life (t_{90}) of gliadin under various conditions were computed from the pseudo–first-order degradation rate constant.

The stability of amoxicillin was studied in SGF (pH 1.2). Samples of amoxicillin (10 mg) were incubated at 37°C in 100 mL of SGF (pH 1.2). At scheduled time intervals, samples were withdrawn and assayed by HPLC using the above-described method in order to measure the amoxicillin concentration.[16]

In Vivo Evaluation of Gastric Mucoadhesion

The in vivo gastric mucoadhesive property of GNP was evaluated by using RITC-entrapped nanoparticles of different gliadin concentration in albino rat stomach as described previously.[18] Albino rats (Sprague-Dawley strain, 200–300 g) of either sex were fasted for 24 hours before the experiments but were allowed free access to water. RITC-entrapped nanoparticles (10 mg) were placed in a polyethylene tube that had 1 end covered with hydroxypropyl cellulose film.[19] Approximately 10 mg of each type of nanoparticle was orally administered through the polyethylene tube attached to a gastric sonde with 2 mL of water. The gastric sonde was attached to a microsyringe containing 0.2 mL of water. The nanoparticles and water were pushed out through the polyethylene tube and orally administered to conscious rats. After 2.5 hours, all the nanoparticles remaining in the stomach were collected after 3 washings with PBS (pH 7.4) and then centrifuged at 20 000 g for 10 minutes. The resulting residues were dispersed in 0.5 mL of aqueous ethanolic solution for 2 hours at 37°C for complete digestion of nanoparticles. The residues were then filtered and the fluorescence intensity of the filtrate was determined in a fluorescence spectrophotometer (Shimadzu) at an emission wavelength of 578 nm and excitation wavelength of 554 nm). The amount of RITC-entrapped GNP remaining in the stomach was calculated from a calibration curve. The gastric mucoadhesion was expressed as the percentage of nanoparticles remaining in the stomach after perfusion.

In Vitro Growth Inhibition Study

Two previously characterized clinical isolates of *H pylori* were used. NCTC 11637 and 11638 were isolated from a gastric biopsy specimen of a patient (age 50) with nonulcer dyspepsia at the Microbiology Laboratory of Sanjay Gandhi Postgraduate Institute of Medical Sciences (Lucknow, India). The biopsy specimen was homogenized with Brucella broth in a sterile glass homogenizer. One loopful of sample was inoculated on Brucella chocolate agar plate. Colonies formed within 5 to 7 days and were subcultured in liquid broth (supplemented with 5% FCS) and solid media (supplemented with 5% sheep blood). The organism was identified by Gram's staining, colony characteristics, and biochemical tests such as urease and catalase. Identified pure culture was transferred aseptically to the filtered Brucella broth supplemented with 5% FCS and allowed to grow for 5 to 7 days under microaerophilic conditions (80% N_2, 15% CO_2, 5% O_2) at 37°C. The number of bacteria were determined as 1 optical density (OD) unit corresponding to 10^6 colony-forming unit (CFU)/mL bacteria at 600 nm in a Shimadzu UV/Visible spectrophotometer.

To characterize the relative contributions of drug and drug-loaded nanoparticles to growth inhibition, *H pylori* suspensions were subjected to amoxicillin (minimum inhibitory concentration–equivalent [MIC-equivalent] dose), amoxicillin (MIC-equivalent dose) loaded nanoparticles, and placebo nanoparticles (GNP) for 4, 8, and 12 hours. Five mL of Brucella broth was inoculated with 100 mL of stock culture of *H pylori*; a micropipette was used to add appropriate volumes of different formulations to culture vials containing

the *H pylori* strain. All vials were incubated in a candle jar under microaerophilic condition with humidity at 37°C. After incubation of all formulations for 12 hours, the growth inhibition was determined in terms of OD for each time interval at 600 nm using a Shimadzu UV/Visible spectrophotometer. Quantitation of in vitro antibacterial activity of formulations was approached in terms of percentage growth inhibition (GI). The method selected was turbidimetry based on OD measurements.

GI was defined as the ratio of the OD of a given test mixture against that of culture vials containing only *H pylori* (Equation 3).

$$\% GI = \frac{OD\ of\ TOPTP - OD\ of\ TMSTP}{OD\ of\ TOPTP} \times 100 \quad (3)$$

where *TOPTP* is test organism at a particular time period and *TMSTP* is test mixture at same time period.

In Vivo Clearance Study

The bacterial strain used in this study was originally isolated from a human patient with gastric ulcer and adapted to the gastric mucosa of Mongolian gerbils (body weight: 50–60 g) by 4 serial passages.[9] Six-week-old male specific pathogen-free Mongolian gerbils were purchased from Banaras Hindu University (Banaras, India) and were maintained under standard laboratory conditions (room temperature, 23° ± 2°C; relative humidity, 55% ± 5%; 12/12 hours light/dark cycle) with free access to a commercial rodent diet and tap water.

H pylori is a human-specific pathogen and causes intense inflammation in conventional experimental animals, the Mongolian gerbil being an exception. Recent studies have indicated that ulcers, intestinal metaplasia, and even adenocarcinoma develop during long-term *H pylori* infection in the animal.[20] The gerbil model may be valuable not only in elucidating *H pylori*–induced neoplasia but also in evaluating virulence factors in vivo, in which a shorter-term model would be preferable.

Six animals each were assigned to 3 groups and were inoculated with 1 mL culture broth via intragastric gavage after fasting for 24 hours. Each dose contained $10^{6.48}$ CFU of *H pylori*. The Institutional Animals Ethical Committee of Dr. Hari Singh Gour University approved the study, which was performed following the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

Fourteen days after infection, amoxicillin was orally administered once a day for 3 consecutive days at a dose of 1, 4, 10, or 40 mg/kg in the form of GNP or amoxicillin suspension. Placebo GNP, used as a control, were administered in the same manner. One day after administration of the final dose, the Mongolian gerbils were killed and the stomachs were removed. Each stomach was homogenized with Brucella broth (3 mL/stomach), and serial dilutions were plated on modified Skirrow's medium. The plates were incubated for 4 days at 37°C under microaerobic conditions in GasPak (BD Diagnostic Systems, Sparks, MD). The viable cell counts for each stomach were calculated by counting the number of colonies on the agar plates. The colonies were identified as *H pylori* by morphology and urease activity. The number of colonies per plate was counted and expressed as log CFU per gastric wall. The advantage of this evaluation method is that errors caused by sampling site variation can be avoided because the whole stomach is used to determine the bacterial cell count.

Statistics

The difference between the control-treated and the amoxicillin-treated groups in bacterial counts of gastric wall were statistically analyzed by one-way analysis of variance with post-test (Dunnett's multiple comparison test). Statistically significant differences between groups were defined as $P<.05$. Calculations were performed with the GraphPad Instat Software Program (GraphPad Software Inc, San Diego, CA).

Results and Discussion

Particle Size and Morphology

GNP have an average particle size range of 392 ± 20 nm to 285 ± 44 nm with positive zeta potential at maximum and minimum gliadin concentration (Table 1). At higher concentrations, aggregates of irregular and nanosize particles were obtained. At lower concentrations, nanosize particles with high polydispersity were obtained. At 100 mg concentration (Table 1), nanosize particles with low polydispersity were observed; it was considered the optimized concentration. At higher concentrations of gliadin, the ethanolic solution was dispersed into numerous fine droplets, which easily coalesced into large viscous droplets producing larger nanoparticles.

The average zeta potential of drug-loaded GNP was 26.6 ± 0.8 mV. The surface morphology of GNP as investigated by SEM revealed a spherical shape with a smooth surface (Figure 1).

Percentage Drug Entrapment and Drug Release Studies

Table 1 shows the effect of initial drug loading on the percentages of payload and entrapment efficiency of the GNP. The amoxicillin entrapment efficiency was found to be high if the

Table 1. Process Variable and Characterization of Gliadin Nanoparticles (n = 3)*

Process Variables	Gliadin Concentration (mg)	Amount of Amoxicillin Used in Nanoparticles (mg)	Observation					
			Particle Size (nm)	Morphology	% Nanoparticles Yield (mg)	% Payload	% Entrapment Efficiency	% Muco-adhesion
Gliadin concentration (mg)	200	80	392 ± 20	Ag, I	70.25 ± 12	60.74 ± 6.2	67.24 ± 4.4	82 ± 4
	150		355 ± 18	Ag, I	71.26 ± 23	60.46 ± 4.4	68.12 ± 6.4	78 ± 3
	100		312 ± 12	Sp, Lp	72.52 ± 26	61.52 ± 2.2	66.54 ± 3.8	72 ± 4
	50		285 ± 44	Sp, Hp	70.96 ± 14	61.34 ± 2.8	68.62 ± 2.6	68 ± 3
Amount of amoxicillin (mg)	150	100	356 ± 18	Ag, I	70.2 ± 16	74.76 ± 2.4	80.25 ± 5.2	-
		80	354 ± 12	Ag, I	72.4 ± 12	50.54 ± 3.2	67.82 ± 4.6	-
		60	348 ± 46	Sp, Hp	71.2 ± 10	31.54 ± 5.2	56.28 ± 5.6	-
		40	354 ± 42	Sp, Hp	70.4 ± 14	15.44 ± 4.2	41.46 ± 3.8	-

*Ag indicates agglomerates; I, irregular; Sp, spherical; Lp, low polydispersity; Hp, high polydispersity.

initial loadings were high. Thus, 100 mg of initial loading resulted in GNP with 80.25 ± 5.2%, whereas 40 mg of initial loading resulted in only 41.46 ± 3.8% entrapment (Table 1). Moreover, the payload was also increased with initial drug loading (Table 1). The payload and entrapment efficiency of formulations were not affected by increasing gliadin concentration. Also, there were no significant changes in particle size and yield when the initial drug loading was increased.

The release pattern of amoxicillin from GNP with various gliadin concentrations is shown in Figure 2A. The drug release rate mainly depended on the gliadin concentration. With increasing gliadin concentration, the release rate of amoxicillin from nanoparticles decreased drastically. Also shown in Figure 2 is the effect of pepsin on drug release rate from drug formulations of different gliadin concentrations. The release rate was faster from all the formulations in the presence of pepsin (Figure 2A, dotted line), because the pepsin partially digested the gliadin. The initial burst release of amoxicillin from GNP at the initial stage resulted from the dissolution of drug crystals on the surface of nanoparticles (Figure 2B).

The effect of initial drug loading on the drug release is shown in Figure 2B. The release rate of amoxicillin remains high with high drug loading and decreases as the loading decreases (Figure 2B). With low drug payloads, the drug particles are widely distributed inside the matrix and separated from each other by the gliadin binder. The drug particles are less likely to come into contact with the dissolution medium, so conditions for rapid dissolution of the drug from the matrix are less favorable. In the case of high drug loading, the drug particles within the GNP are in close proximity with each other, so the particles are more likely to come into contact with the dissolution medium. The dissolution produces channels throughout the matrix, facilitating the rapid erosion of the drug. The higher surface concentration of the drug is believed to exert little effect on the release profile, as most of the surface drug is liberated during the initial burst.

In Vivo Evaluation of Gastric Mucoadhesion

Mucoadhesion involves different kinds of interaction forces between mucoadhesive materials and the mucus surface; these include electrostatic attraction, hydrogen bonding, Van der Waals forces, mechanical interpenetration, and entanglement. Many methods have been used to evaluate these interactions in vitro and in vivo. A fluorescence probe method was used to measure in vivo mucoadhesive capacity of developed formulations. RITC entrapment efficiency was 56.4 ± 4.2%, and after 2.5 hours, the amount of RITC released from the GNP was 9.6 ± 2.2%. The above points were also considered in the measurement of mucoadhesion.

The mucoadhesion capacity increased with an increasing gliadin concentration. All the formulations showed more than 65% of mucoadhesive capacity (Table 1). In formulations with higher gliadin concentrations, the amount of nanoparticles remaining was calculated to be 82 ± 4%, which represented a strong mucoadhesive propensity and specificity (Table 1). Gliadin protein is rich in neutral amino acids and lipophilic residues. Neutral amino acids can promote hydrogen-bonding interactions with the mucosa, whereas the lipophilic components can interact with the biologic tissue by hydrophobic interactions.[15]

The method of Shah et al[21] was used to conduct the in vitro permeation studies of amoxicillin in SGF through gastric mucin and gastric fluid. They reported that the permeability coefficient of amoxicillin was 5.5×10^{-6} cm per second^{-1} through gastric fluid and 2.3×10^{-6} cm per second^{-1} through gastric mucin. Assuming that the permeability coefficient of amoxicillin is approximately the same in gastric mucus, the drug would penetrate a 200-µm layer in 2.4 hours. In vivo mucoadhesion study revealed that more than 80% of the GNP remained in the stomach after 2.5 hours. So it would be expected that amoxicillin released from the GNP would penetrate the mucus and effectively eradicate and kill the *H pylori* in stomach mucosa.

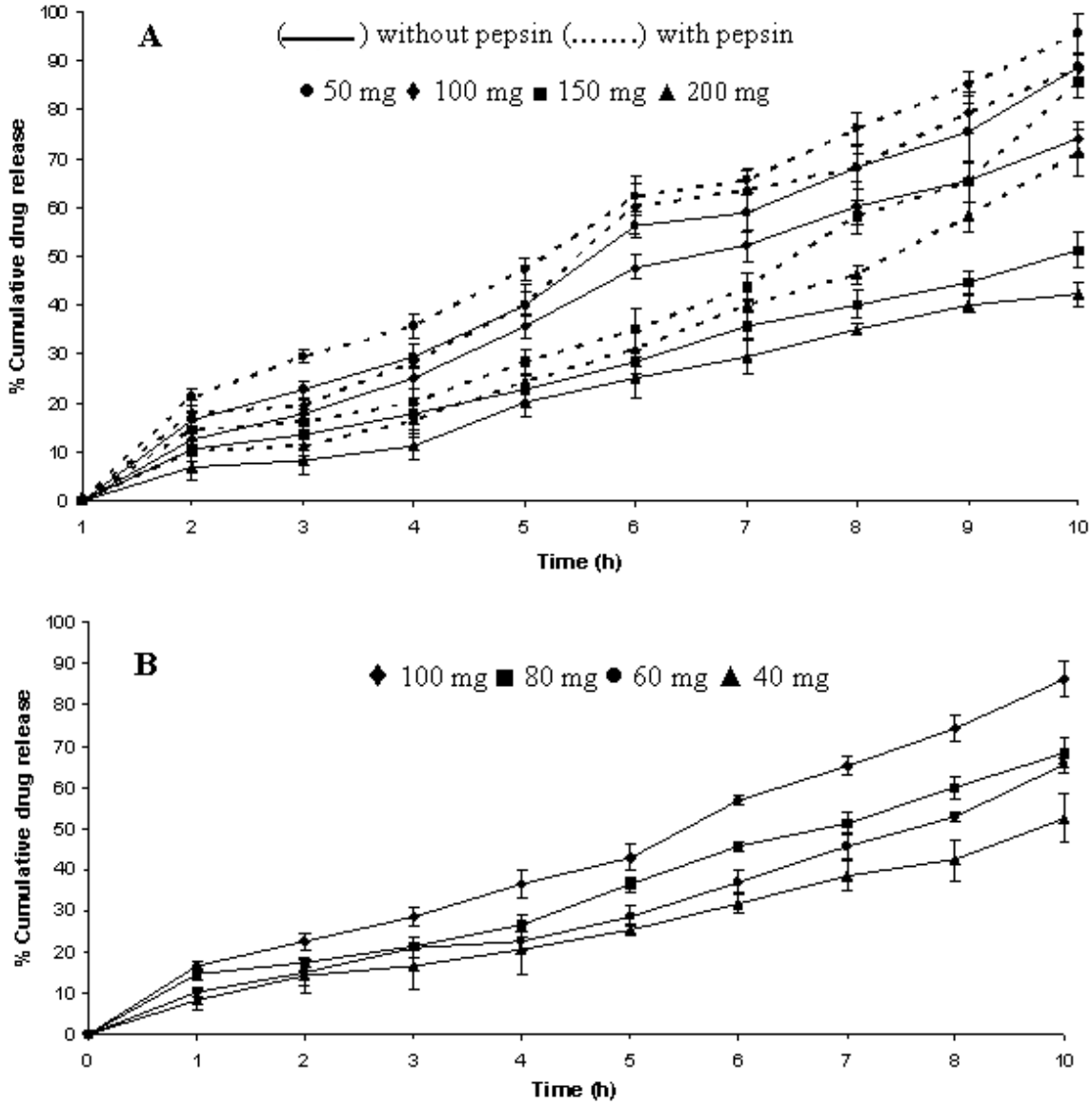

Figure 2. Effect of (A) gliadin concentration and (B) initial drug loading on amoxicillin release from AGNP in SGF (pH 1.2) (n = 3).

Formulations with 100 mg of gliadin and 80 mg of amoxicillin showed a spherical shape with a low polydispersity index, a better and more consistent drug release profile, and satisfactory mucoadhesive properties (72 ± 4%). This formulation, coded as AGNP, was prepared at an above-optimal concentration and used for further in vitro GI and in vivo clearance studies.

Stability Studies of Gliadin and Amoxicillin

The first-order rate constant (k), half-life (t_{50}), and shelf life (t_{90}) were calculated from the slope of log percent remaining vs time. The k value, half-life, and shelf life of gliadin at pH 1.2 were 0.01568 h^{-1}, 44.17 hours, and 6.80 hours, respectively. Gliadin was comparatively stable in SGF (pH 1.2). In SGF (pH 1.2), amoxicillin degradation was much quicker and displayed strict first-order kinetics. The pseudo–first-order constant, half-life, and shelf-life values were 4.2×10^{-2} h^{-1}, 16.5 hours, and 2.54 hours, respectively. After 16 hours, ~50% of the starting drug amount was degraded.

In Vitro GI Study

The results of percentage GI study could be arranged in order of percentage GI performances: amoxicillin > AGNP > GNP. The maximum percentage GI of placebo GNP after 12 hours was found to be 5%. The results clearly indicated that the polymer does not exhibit any antimicrobial activity.

To characterize the in vitro efficacy of amoxicillin and the AGNP formulation, the turbidity of *H pylori* was measured after incubation with AGNP for up to 12 hours. The time required for complete GI by amoxicillin and AGNP was compared. After incubation with AGNP for 4 hours, bacteri-

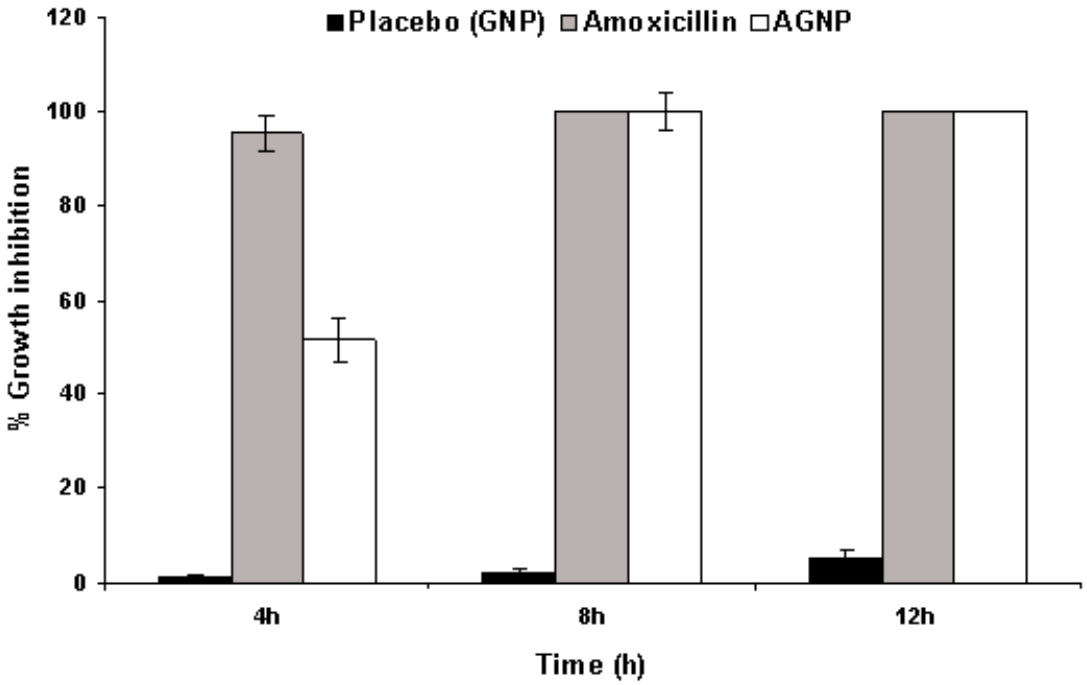

Figure 3. Percentage GI of formulations.

al growth was reduced to 51.6 ± 4.6% (Figure 3). Within 4 hours, amoxicillin completely inhibited the *H pylori* growth. Continued incubation of *H pylori* whole cells for up to 8 hours in the presence of AGNP completely inhibited the bacterial growth. Although an MIC-equivalent dose was used in both AGNP and amoxicillin, the controlled delivery of AGNP meant that the microorganisms were exposed to less of the drug. Thus, the time required for complete inhibition was less for amoxicillin than for AGNP because of the direct exposure of the amoxicillin to the *H pylori*.

Amoxicillin is one of the most active and predictable antimicrobial agents against *H pylori* and also has rapid bactericidal activity compared with the various antibiotics and antiulcer agents.[22] Amoxicillin has an MIC_{90} of 0.12 mg/L against clinical isolates of *H pylori* and is more active than erythromycin, roxithromycin, and azithromycin (MIC_{90} 0.25 mg/L for each). Amoxicillin inhibits transpeptidase and stops the synthesis of peptidoglycan, thereby weakening the cell wall and making *H pylori* more susceptible to death due to bursting.

In Vivo Clearance of H pylori

The in vivo clearance data of *H pylori* after multiple administration of AGNP formulation or the amoxicillin suspension under fed conditions is presented in Table 2. The mean bacterial count after oral administration of the amoxicillin suspension decreased as the dose of amoxicillin increased, but complete clearance of *H pylori* was not obtained even with the highest dose (Table 2). Although amoxicillin completely inhibited the *H pylori* growth in vitro (100% GI, Figure 3) within 4 hours, it could not completely eradicate the *H pylori* in vivo. This is because of the short residence time of amoxicillin suspension in the stomach and the low concentration of amoxicillin reaching the bacteria under the gastric mucus layer. Most antibacterial agents have low MICs against *H pylori* culture. However, single antibiotic therapy with conventional antimicrobial formulations is not effective for the eradication of *H pylori* infection in vivo.

The mean bacterial count after 3 days of treatment with AGNP (amoxicillin dose of 1.0 mg/kg) was found to be log CFU 5.84 ± 0.24, which was significantly lower than amoxicillin suspension (log CFU 7.98 ± 0.56, Dunnet's test). Complete clearance of *H pylori* (clearance rate, 100%) was observed after the administration of AGNP at doses of 10 and 40 mg/kg. AGNP with an amoxicillin dose of 1 mg/kg provided the same clearance rate (33%) as that of amoxicillin suspension at a dose of 10 mg/kg. These results demonstrate that the AGNP provided 10 times greater anti–*H pylori* activity than the amoxicillin suspension. The dose required for complete eradication is significantly less in mucoadhesive nanoparticles than amoxicillin.

The present study showed that amoxicillin-bearing GNP resided in the stomach for a longer time than amoxicillin. The in vivo results provide ample evidence that the topical action of amoxicillin on the gastric mucosa plays an important role in the clearance of *H pylori*.

CONCLUSION

Qualitative mucoadhesive study suggested that the adhesion of nanoparticles to the stomach and the resulting higher drug

Table 2. Effect of Repetitive Oral Administration of Amoxicillin Suspension and AGNP Formulation Against Gastric Infection Caused by *Helicobacter pylori* in Mongolian Gerbils (n = 6)

Formulations	Dose (mg/kg)†	Clearance Rate (no of Mongolian Gerbils Cleared of Infection/Total no), (%)	Mean Bacterial Counts‡ (Log CFU ± SD)
Placebo GNP	0	0/6 (0)	8.04 ± 0.14
Amoxicillin	1	0/6 (0)	7.98 ± 0.56§
	4	0/6 (0)	7.24 ± 0.29|
	10	2/6 (33)	5.92 ± 0.47|
	40	4/6 (66)	4.72 ± 0.10|
AGNP	1	2/6 (33)	5.84 ± 0.24|
	4	5/6 (77)	4.42 ± 0.16|
	10	6/6 (100)	ND
	40	6/6 (100)	ND

*AGNP indicates GNP-bearing amoxicillin; CFU, colony-forming unit; GNP, gliadin nanoparticles; ND, not detected (CFU value less than 0.5).
†Once daily for 3 days as amoxicillin.
‡Bacterial counts less than 101.44 were considered to be 101.44 CFU to calculate the mean. Values are mean ± SD.
§$P > .05$ nonsignificant from control's Dunnett's test.
|$P < .01$ significant from control's Dunnett's test.

concentration gradient as well as transport through the mucosa were responsible for the enhancement of local drug concentration at infection sites. The preferential accumulation of nanoparticles in the stomach may be very useful for targeting the antibiotics at the site of infection and possibly also for *H pylori* eradication. It is possible that GNP with uniform gastric distribution could target the *H pylori*–infected sites more effectively and completely eradicate the *H pylori*, which could be of definite therapeutic benefit.

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