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Floating microspheres bearing acetohydroxamic acid for the treatment of *Helicobacter pylori*

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Abstract

This investigation is part of our ongoing effort to develop effective drug delivery systems for the treatment of *Helicobacter pylori* infection using polycarbonate (PC) floating microspheres as drug carriers. In an effort to augment the anti-*H. pylori* effect of acetohydroxamic acid (AHA), floating PC microspheres, which have the ability to reside in the gastrointestinal (GI) tract for an extended period, were prepared by emulsion (O/W) solvent evaporation technique. The effect of PC concentration on the morphology, particle size, entrapment efficiency and drug release rate was studied. In-vitro studies confirmed the excellent floating properties of PC microspheres. In-vitro and in-vivo growth inhibition studies were performed on developed system(s) taking isolated cultures of *H. pylori* and *H. pylori*-infected Mongolian gerbils, respectively. The drug and PC microspheres both showed anti-*H. pylori* activity in vivo, but the required dose of AHA was effectively reduced by a factor of 10 in the case of PC microspheres. In conclusion, the floating microspheres more effectively cleared *H. pylori* from the GI tract than the drug because of the prolonged gastric residence time resulting from the excellent buoyancy of the PC.

Introduction

Helicobacter pylori (*H. pylori*), a prevalent human-specific pathogen, is a causative agent in chronic active gastritis, duodenal ulcers and gastric adenocarcinoma (Forman et al 1994), one of the most common forms of cancer in humans. Although tremendous efforts have been devoted to designing efficient drug delivery systems for *H. pylori* treatment, the clinical applicability of such systems is limited by a number of drawbacks. Some of the important concerns are: (i) the Gram-negative organism *H. pylori* exclusively resides on the luminal surface of the gastric mucosa under the mucus gel layer and hence access for antimicrobial drugs to the site of infection is restricted, both from the lumen of the stomach and from the gastric blood supply, and (ii) conventional drug delivery systems do not remain in the stomach, therefore the antibiotics are not delivered to the site of infection in effective concentrations and in fully active form from the drug delivery systems.

One way to improve the efficacy of eradicating *H. pylori* infection is to deliver the antibiotic locally to the stomach. Better stability and longer residence times allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori*. Topical delivery through gastroretentive systems has been suggested as an approach to overcoming the problems of residence time with antibiotics. Research into gastroretentive drug delivery systems has resulted in the development of several approaches, including the use of a sucralfate–tetracycline complex (Yokel et al 1995), positively charged gelatin microspheres (Wang et al 2000) and chitosan microspheres (Hejazi & Amiji 2002) to prolong the residence time of formulations at infected sites with the aim of complete eradication of *H. pylori*. In previous work, we reported the efficacy of polycarbophil-coated floating microspheres (Umamaheshwari et al 2002), chitosan-conjugated fucose nanoparticles (Umamaheshwari et al 2003a) and cholestyramine microcapsules (Umamaheshwari et al 2003b) with isolated *H. pylori* culture. These formulations significantly inhibited the growth of *H. pylori* in vitro. This work has been extended to prepare floating polycarbonate (PC) microspheres and evaluate their efficacy in *H. pylori* treatment.

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The various buoyant preparations available include hollow microspheres (microballons), granules, powders, capsules, tablets (pills) and laminated films. Most of the floating systems are dominated by single unit formulations (e.g. hydrodynamically balanced systems). A drawback of the system is the high variability of the gastrointestinal (GI) transit time because of the 'all or nothing' emptying processes. A multiple unit floating system (PC microspheres) that can be distributed widely throughout the GI tract, providing the possibility of achieving a longer lasting and more reliable release of drug, has therefore been sought.

An important characteristic of *H. pylori* is its substantial urease activity, which appears to be essential for the survival and pathogenesis of the bacterium. It is thought that hydrolysis of urea by urease generates ammonia to counterbalance gastric acidity, presumably by forming a neutral microenvironment surrounding the bacterium within the gastric lumen. Supporting this hypothesis, it has been shown that *H. pylori* survive at low pH in vitro in the presence of urea and functional urease activity (Meyer-Rosburg et al 1996). Furthermore, isogenic urease-negative mutants of *H. pylori* and of related bacteria, *Helicobacter mustelae*, which lacks urease activity, are unable to colonize the gastric mucosa of mice, ferrets and gnotobiotic piglets (Andrutis et al 1995). In the light of this, we sought to determine the relative contribution of potent urease inhibitor-(acetohydroxamic acid, AHA) loaded PC-formulations in the eradication of *H. pylori* in vitro and in vivo.

Materials and Methods

Materials

The materials used were PC (Polybisphenol A carbonate, M_w 29 000 and M_n 17 000, Aldrich, USA) and AHA (Fluka, USA); methylene chloride, Tween 20, polyvinyl alcohol, methanol, sodium chloride, hydrochloric acid (Loba Chemie, India), brain heart infusion agar, yeast extract, fetal calf serum and *Campylobacter* selective component (Skirrow supplement, SR 69) (Himedia, India). All other materials were of AR grade and double distilled water was used for all experiments.

Formulation of PC microspheres

Hollow PC microspheres loaded with AHA were prepared by the solvent evaporation process reported earlier (Thanoo et al 1993; Joseph et al 2002). Briefly, various concentrations of PC solution in dichloromethane were mixed well with the required amount of the drug and this pasty, flowable mass was introduced into 50 mL of aqueous saline phase containing 0.04% polyvinyl alcohol (PVA) and 10% methanol. The system was stirred using a half-moon paddle stirrer at 300 rev min^{-1} at room temperature for 2–3 h. The drug-loaded floating PC microspheres formed were collected by centrifugation, washed three times with deionized water and dried in a hot air oven at 60 °C.

Mean diameter and morphology

The volumetric size distribution of the microspheres was determined by laser light scattering with a CILAS-1064 particle size analyzer (CILAS Instruments, France) and standard deviations were calculated from the cumulative distribution curve. The surface morphology and internal structure were visualized by scanning electron microscopy (SEM, Jeol 1804, Japan). The samples for SEM were prepared by lightly sprinkling the microsphere powder on a double adhesive tape stuck to an aluminium stab. The stabs were then coated with gold to a thickness of about 300 Å using a sputter coater. The samples were then randomly scanned and photographs were taken.

The hollow structure of the microspheres was estimated by measuring the particle density (P_p) by a liquid displacement method. The weighed microspheres were immersed in an aqueous solution (0.02%, w/v) of Tween 20 in a pycnometer (25 mL) at 20 °C.

The microspheres were also characterized by their porosity (ϵ) using the equation:

$$\epsilon = (1 - P_p/P_t) \times 100$$

where P_t is the true density measured using a helium-air pycnometer model 1302, (Micrometrics Instrument Co., USA).

X-ray diffractometric analysis was carried out using a Philips 1032 PW 1820 diffractometer (Philips, The Netherlands) with graphite monochromator Cu-K_2 radiation. Samples of AHA, PC, AHA-loaded PC microspheres and the physical mixture of PC and AHA were analysed.

Entrapment efficiency

Entrapment efficiency was determined by dissolving microspheres in 0.1 M hydrochloric acid followed by 10 mL of methylene chloride, and the digested homogenate was centrifuged at 3000 rev min^{-1} for 3 min. After evaporation of dichloromethane, the solution was filtered and the filtrate was assayed for AHA colorimetrically at 503.5 nm using a Shimadzu 1601 UV/visible spectrophotometer.

In-vitro buoyancy studies

The floating microspheres (300 mg) were spread over the surface of the dissolution medium (simulated gastric fluid, SGF, pH 1.2) containing Tween 20 (0.02% w/v) that was agitated by a paddle rotated at 100 rev min^{-1}. After agitation for a predetermined time interval, the microspheres that floated over the surface of the medium and those settled at the bottom of the flask were recovered separately. After drying, each fraction of the microspheres was weighed and the buoyancy of the microspheres was calculated by the following equation:

$$\text{buoyancy}\,(\%) = Q_f/(Q_f + Q_s)$$

where Q_f and Qs are the weight of the floating and the settled microspheres, respectively.

In-vitro drug release

The drug release studies of floating microspheres were carried out using a USP dissolution apparatus I. The respective formulations were spread over the surface of 900 mL of SGF (pH 1.2), rotated at 100 rev min^{-1} and thermostatically controlled at 37 °C. Perfect sink conditions prevailed during the drug dissolution tests. The samples were withdrawn from the dissolution vessel at suitable intervals and were assayed colorimetrically at 503.5 nm for AHA using a Shimadzu UV/visible spectrophotometer.

In-vitro growth inhibition studies

Two previously characterized clinical isolates of *H. pylori* were used. NCTC 11637 and 11638 were isolated in 1999 from patients with gastric ulcers. The isolation was performed in the Microbiology Laboratory, Rajaji Government Hospital, Madurai, India. The isolated samples were grown in the selective media containing brain heart infusion agar 1%, yeast extract 0.25%, fetal calf serum 10% and *Campylobacter* selective component 0.4%. To suppress the growth of endogenous or exogenous contaminating bacteria, selective media are required to improve the isolation of *H. pylori* from biopsy samples. All strains were grown in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2) at 37 °C.

In general, the methods described by McGowan et al (1994) were used to assess the in-vitro efficacy of drug and formulations on the growth inhibition of *H. pylori*. Briefly, bacteria were harvested from culture plates and suspended in normal saline (150 mmol of NaCl per litre, pH 7.2) to yield a final suspension of approximately 10^9 colony forming units (CFU) mL^{-1}. Bacterial suspensions were then diluted 1:10 (final concentration, 15 mmol of NaCl per litre). The diluted suspensions were incubated in 100 mmol of citric acid:HCl buffer per litre (pH 2) with or without urea (5 to 50 mmol) in a microaerobic environment. After 30 min of incubation, serial dilutions were made in normal saline (pH 7.2), and 0.1 mL of an appropriate dilution was plated onto blood agar plates and incubated at 37 °C for 72–96 h in a microaerophilic environment to enumerate viable bacteria (CFU mL^{-1}).

AHA was used at a final concentration of 7 mM (7 mM is approximately twice the reported MIC_{50} for *H. pylori* urease; Mobley et al 1988). Briefly, whole cell suspensions of *H. pylori* were incubated with AHA (7 mM)/PC-3 formulations (7 mM equivalent dose)/placebo microspheres for 30 min in brucella broth containing 10% fetal bovine serum in a microaerophilic environment prior to exposure of the culture to an acidic environment (100 mmol of citric acid:HCl buffer per litre, pH 2) for an additional 30 min of incubation. At the end of the incubation, serial dilutions were made to enumerate the viable bacteria as described above.

Time course of growth inhibition

Suspensions of *H. pylori* were incubated for various time intervals in a microaerophilic environment in the presence of AHA (7 mM)/PC-3 formulations (7 mM equivalent AHA dose)/placebo microspheres. At the end of the incubation, serial dilutions were made to enumerate the viable bacteria as described above.

In-vivo clearance of *H. pylori*

The bacterial strain used in this study was originally isolated from a human patient with a gastric ulcer and was adapted to the gastric mucosa of Mongolian gerbils (body weight 50–60 g) by four serial passages. 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 h light/dark cycle) with free access to a commercial rodent diet and tap water. Six animals were assigned to each of three groups and were inoculated with 1 mL of culture broth via intragastric gavage after fasting for 24 h. Each dose contained $10^{9.48}$ CFU of *H. pylori*. The Institutional Animals Ethical Committee of Dr Hari Singh Gour University approved the study. The studies were carried out using the guidelines of the Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Fourteen days after infection, AHA was administered orally once a day for three consecutive days at a dose of 1, 3, 10 or 30 mg kg^{-1} in the form of PC microspheres (PC-3) bearing AHA or AHA solution. Placebo PC microspheres, which were used as the control, were administered in the same manner. One day after administration of the final dose, the Mongolian gerbils were killed and their stomachs were removed. Each stomach was homogenized with brucella broth (3 mL per stomach) and serial dilutions were plated on modified Skirrow's medium. The plates were incubated for four days at 37 °C under microaerophilic conditions in GasPak jars. The viable cell counts for each stomach were calculated by counting the number of colonies on the agar plates. Colonies were identified as *H. pylori* by morphology and urease activity. The number of colonies per plate was counted and expressed as the log colony-forming units (log CFU) per gastric wall.

Statistics

Differences in bacterial counts in the gastric wall between the control-treated and the AHA-treated groups were statistically analysed by one-way analysis of variance (ANOVA) with post test (Dunnett's multiple comparison tests). Statistically significant differences between groups were defined as $P < 0.05$. Calculations were performed with the GraphPad-Instat Software Program (GraphPad-InStat Software Inc., San Diego).

Results and Discussion

Mean diameter and morphology

Floating PC microspheres bearing AHA were obtained by employing emulsion solvent evaporation technology

(Thanoo et al 1993; Joseph et al 2002). Initially, the organic phase containing PC was emulsified in a large volume of aqueous phase containing 0.04% PVA. The rapid evaporation of methylene chloride leads to precipitation of the polymer on the emulsion droplets, producing hollow microspheres. The microspheres prepared by the above technique were spherical in shape with a hollow cavity.

The SEM photographs (Figures 1 and 2) show the surface morphology and thin cross-sectional view of floating PC microspheres, respectively. Figure 2 shows the characteristic internal hollow cavity of the microsphere, which was enclosed within the rigid shell, constructed of drug and polymer. Microsphere diameters ranged from 240 to 288 μm, as observed in Table 1. It is obvious that an increase in the PC concentration causes an increase in the viscosity of the emulsion, resulting in larger microsphere diameters (Table 1). At higher concentrations of PC, the polymeric solutions were dispersed into numerous fine droplets, which easily coalesced into larger viscous droplets during the evaporation of dichloromethane out of the droplets, producing larger microspheres. The stirring efficiency might have been reduced because of the increased viscosity at higher PC concentrations. Slight aggregation of particles resulted because of increased frequency of collision when the PC concentration was increased. The data on entrapment efficiency of AHA in microspheres of different PC concentrations are given in Table 1. The entrapment efficiency increased with increasing PC concentration. X-ray diffraction studies confirmed the amorphous nature of the drug within the PC microspheres (data not shown).

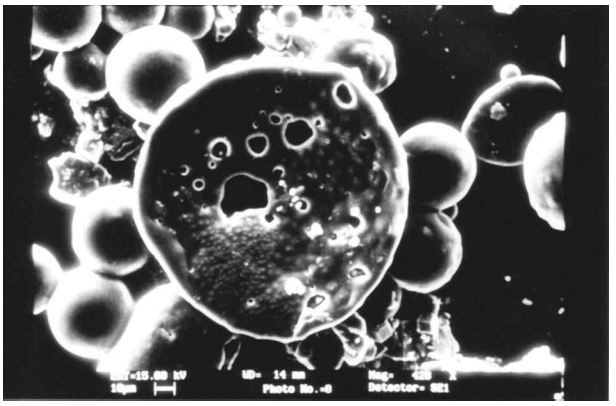

Figure 1 Scanning electron micrograph of surface morphology of PC microspheres.

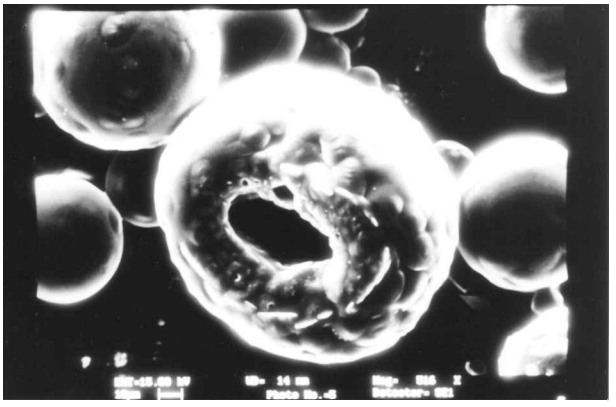

Figure 2 Scanning electron micrograph of cross-section of PC microspheres. Photograph shows the inner hollow structure of microspheres.

In-vitro buoyancy studies

In-vitro floating behaviour was investigated in an acidic medium containing a small amount of surfactant (Tween 20) and agitated with a paddle at 100 rev min^{-1} to simulate the wetting action of gastric fluid under movement. The in-vitro floating test clearly showed that the PC-1 and PC-2 formulations were floating after 12 h of testing, which could be attributed to their pores and cavities (Figures 1 and 2). The percentage buoyancies of the PC-3 and PC-1 formulations were 74 and 85%, respectively. The microspheres with higher concentrations of polymer (PC-3) were less buoyant than those with lower concentrations of polymer. This difference may be attributed to an increase in the density of microspheres with increasing polymer concentration (Table 1). The relative density of PC-3 formulations was higher (0.910 g cm^{-3}) than in PC-1 formulations (0.710 g cm^{-3}). The porosity of microspheres remains less at higher polymer concentrations and increases as the polymer concentration decreases. The microspheres appeared to be hollow and porous presumably because of the rapid escape of the volatile solvent from the polymer matrix. At higher polymer concentrations, the viscosity of the polymer solution increased, the higher viscosity slowed down solvent evaporation and hence reduced porosity, resulting in decreased percentage buoyancy. Floating up to 10 h was considered to be a satisfactory result. In order to understand buoyancy in a better way, further work (in-vivo floating studies) is required.

In-vitro drug release

The effect of polymer concentration on drug release rate was observed in SGF (pH 1.2). The drug release rate clearly depended on the polymer (PC) concentration in the formulated system (Figure 3). The drug release rate was significantly reduced with PC concentration. AHA is a highly water-soluble drug and it was present in amorphous form within the formulations. In spite of its amorphous nature, the drug release rate was not enhanced as a result of the hydrophobic and insoluble nature of the polymer. The burst release was higher in the PC-1 than in the PC-3 formulation. At lower concentrations of polymer (PC-1), the surface of the microspheres was rough and the drug crystals seen on the surface of the microspheres are probably responsible for the burst effect. The porosity of the PC-1 formulations was higher than in the PC-3

Table 1 Physical properties of PC formulations (n = 3).

Drug:PC ratio	Code	Mean particle size (μm)	Relative density (g cm^{-3})	Porosity (%)	Buoyancy[a] (%)	Entrapment (%)
1:2	PC-1	240.56 ± 4.8	0.710 ± 0.04	65.4 ± 8.9	85 ± 9.2	83.7 ± 2.7
1:4	PC-2	272.69 ± 6.8	0.872 ± 0.06	54.6 ± 9.3	80 ± 6.3	85.3 ± 2.6
1:6	PC-3	287.88 ± 3.5	0.910 ± 0.02	48.4 ± 4.1	74 ± 5.2	88.3 ± 4.5

Values are mean ± s.d. [a]After 12 h.

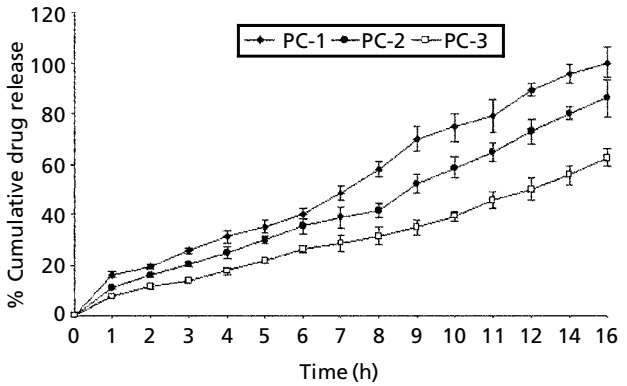

Figure 3 In-vitro drug release of AHA from various PC formulations in simulated gastric fluid (pH 1.2) at 37 °C. Values are mean ± s.d. (n = 3).

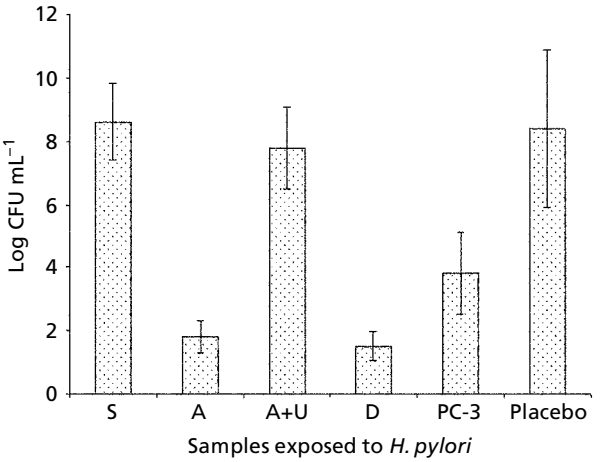

Figure 4 Effects of AHA on *H. pylori* (72-h fresh subculture) growth. Preincubation of *H. pylori* with normal saline pH 7.2 (S); citric acid/HCl buffer pH 1.2 (A); citric acid/HCl buffer with 5 mM urea (A + U); AHA, 7 mM (D); AHA (7 mM) bearing PC-3 formulations (PC-3); placebo PC-3 microspheres (placebo). Serial dilutions were made and plated onto blood agar plates for up to 72 h and subjected to viable counts. Values are mean ± s.d. (n = 3).

formulations. The contact of dissolution medium with the drug is therefore highly favourable for rapid dissolution of the drug from the matrix. PC-1 and PC-2 formulations showed slower drug release rate up to 8 h, thereafter the rate was high up to 15 h. Within 15 h, 90% of the drug was released from PC-1 formulations. In-vitro buoyancy studies showed that the PC-1 and PC-2 formulations were floating > 12 h. During floating (up to 8 h) the contact of dissolution medium with drug was less favourable for dissolution of the drug from the matrix.

In-vitro growth inhibition studies

To determine the ability of *H. pylori* to survive exposure to acid, *H. pylori* strain (exposed to 50 mM urea to induce synthesis of urease) was exposed to acid in the presence or absence of 5 mM urea. At pH 7.2 (saline) in the absence of urea, all microorganisms survived (log 10 CFU 8.6 ± 0.14); however, at pH 2 in the presence of urea, the results (log 10 CFU 7.8 ± 0.12) are similar to that in the presence of saline, while at pH 2 in the absence of urea, survival of *H. pylori* was reduced significantly (log 10 CFU 1.8 ± 0.02, Figure 4).

H. pylori cells with urease activity survived in an acid environment, but only when urea was present at 5 mM, thus confirming the observations of previous investigations (Eaton et al 1991). To characterize the relative contributions of AHA and AHA-loaded formulations to growth inhibition, we subjected suspensions of *H. pylori* to 7 mM AHA, AHA-(7 mM) loaded formulations and placebo microspheres for 30 min. Subsequently, bacteria were exposed to an acidic environment (100 mmol of citric acid:HCl buffer per litre, pH 2) in the presence of urea (5 mM).

After 30 min of exposure to AHA (7 mM), which inhibited > 90% of growth (Figure 4), there was a 6-log reduction in bacterial survival. Preincubation of *H. pylori* with PC-3 formulations (7 mM) resulted in a 3- to 4-log reduction in bacterial survival on exposure to acid (Figure 4). To check any antimicrobial activities of the constitutive ingredients, placebo microspheres were taken for the studies. Preincubation of *H. pylori* with placebo microspheres (7 mM) had no growth inhibition effect, as shown in Figure 4. The results confirmed that the polymer had no antimicrobial activity.

To characterize the growth inhibition of AHA and AHA-loaded formulations, we assessed the viable count after exposure of *H. pylori* to these formulations for up to

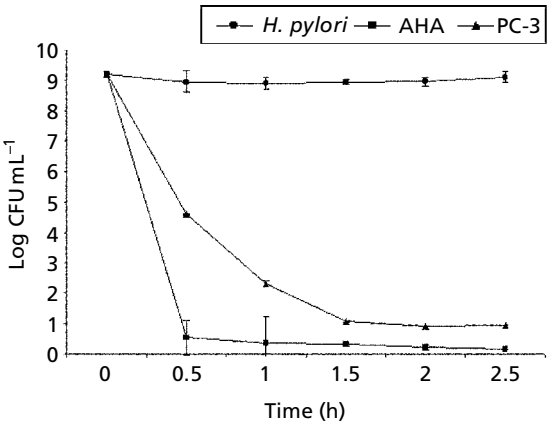

Figure 5 Effects of AHA and AHA-loaded formulations on the time course of growth inhibition in whole cell suspensions of *H. pylori* (72 h fresh subculture). Values are mean ± s.d. (n = 3).

150 min. After incubation with formulations for 30 min at pH 7.2, urease activity was reduced to approximately 50% of the initial activity. In contrast, a freely diffusible inhibitor AHA inhibited over 95% of urease activity after 30 min (Figure 5). Continued incubation of *H. pylori* whole cells for up to 60 min in the presence of formulations significantly reduced the viability. Although the formulation represents the same amount of AHA as drug, (7 mM) the microorganisms were exposed to less concentration of AHA due to controlled drug delivery from formulations. In 30 min, 3.7% of entrapped drug was released from the formulations (Figure 3) and this was not sufficient to inhibit the microorganisms completely. Thus, the time required for complete inhibition was more with the formulations than with the drug. AHA, a potent specific urease inhibitor, remarkably inhibits ammonia production and LDH release in a dose-dependent manner (El Nujumi 1991). AHA is the most widely exploited example of the urease inhibitor class. Also, *H. pylori* is known to have chemotactic activity towards urea and bicarbonate. AHA inhibits these mechanisms (El Nujumi 1991).

In-vivo clearance of *H. pylori*

In general, the method described by Nagahara et al (1998) is used to assess the in-vivo usefulness of a floating dosage form, i.e. PC-3 microspheres, for the eradication of *H. pylori*. In-vivo evaluation of AHA microspheres was carried out with an animal model, Mongolian gerbils infected with human *H. pylori*. The advantage of this evaluation method is that errors due to sampling site variation can be avoided because the whole stomach is used to determine the bacterial cell count.

H. pylori is a human-specific pathogen that 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 (Ikeno et al 1999). 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 will be preferable.

In-vivo clearance data of *H. pylori* after multiple administrations of AHA microspheres or AHA solution under fed conditions (AHA dose 1, 3, 10 and 30 mg kg^{-1}) is presented in Table 2. The mean bacterial count after oral administration of AHA solution decreased as the dose of AHA increased, but complete clearance of *H. pylori* was not obtained even with the highest dose. Although AHA showed maximum percentage growth inhibition in vitro, it did not show complete eradication of *H. pylori* in vivo. This difference is because of the short residence time of AHA in the stomach and the low concentration of AHA reaching the bacteria under the gastric mucous layer.

The mean bacterial counts after three days of treatment with AHA microspheres (dose 1.0 mg kg^{-1}) were significantly lower (log CFU 4.21 ± 0.10) than for AHA solution using the same dose (log CFU 9.21 ± 0.56). Complete clearance of *H. pylori* (clearance rate 100%) was observed in the case of AHA microspheres with AHA doses of 10 and 30 mg kg^{-1}. The AHA microspheres (dose 1 mg kg^{-1}) provided the same clearance rate (33%) as the AHA solution (dose 10 mg kg^{-1}). These results reveal that the AHA microspheres provide 10 times greater anti-*H. pylori* activity than the AHA solution. The AHA dose required for complete eradication is less in the case of floating microspheres than for the drug.

In this study, we found that AHA resided in the stomach for a longer period of time when it was administered in the form of floating microspheres. The AHA microspheres provided greater anti-*H. pylori* activity because of the prolonged residence time. Considering in-vitro growth inhibition studies and in-vivo results, it is amply evident that the topical action of AHA on the gastric mucosa plays an important role in the clearance of *H. pylori*. The absorption of an antibiotic into the mucus through the mucus layer (from the gastric lumen) is believed to be a more effective strategy for *H. pylori* eradication than absorption through the basolateral membrane (from blood) (Kimura et al 1995).

Conclusions

From all of the experiments performed it can be concluded that the developed system can be successful in the treatment of *H. pylori*. The system not only curtails or alleviates the shortcomings of conventional drug delivery systems, it can also deliver the antimicrobial effect to the infected mucosal cell lines. It is possible that floating PC microspheres with uniform gastric distribution can target the *H. pylori*-infected sites more effectively and could optimize antibiotic monotherapy of *H. pylori*, which could be of definite therapeutic benefit. Furthermore, in order to confirm the efficiency of developed formulations, in-vivo buoyancy and pharmacokinetic studies have been planned.

Table 2 Effect of repetitive oral administration of AHA solution and PC-3 formulations against gastric infection caused by *H. pylori* in Mongolian gerbils (n = 6).

Formulations	Dose (mg kg^{-1})[a]	Clearance rate (no. of Mongolian gerbils cleared of infection/total no. (%))	Bacterial mean counts (log CFU ± s.d.)[b]
Control[c]	0	0/6 (0)	9.27 ± 0.01
AHA solution	1	0/6 (0)	9.21 ± 0.56^{ns}
	3	0/6 (0)	9.01 ± 0.29^{ns}
	10	2/6 (33)	$7.42 \pm 0.47^{**}$
	30	3/6 (50)	$6.48 \pm 0.10^{**}$
PC-3 formulations	1	2/6 (33)	$4.21 \pm 0.10^{**}$
	3	4/6 (67)	$2.66 \pm 0.05^{**}$
	10	6/6 (100)	ND[d]
	30	6/6 (100)	ND[d]

[a]Once daily for three days as AHA. [b]Bacterial counts less than $10^{1.44}$ were considered to be $10^{1.44}$ to calculate the mean. [c]Control was placebo PC-3 microspheres. [d]ND, not detected. $^{**}P < 0.01$ significant difference from control, $^{ns}P > 0.05$ non-significant difference from control. Statistical tests were done by one-way of analysis of variance with post test (Dunnett's multiple comparison test).

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