In vitro Evaluation of Amoxicillin Trihydrate and Clarithromycin-Loaded Mucoadhesive Microspheres for H. pylori Eradication

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Abstract

Purpose: To evaluate in vivo H. pylori clearance efficacy of formulated mucoadhesive microspheres of amoxicillin trihydrate and clarithromycin.

Methods: Amoxicillin trihydrate and clarithromycin mucoadhesive microspheres were prepared by solvent evaporation method using Carbopol 974P, HPMC K4M and Eudragit RS 100. In vivo clearance efficacy of the microspheres was evaluated in a Wistar rat model after induction of H. pylori infection. Amoxicillin and clarithromycin-loaded microspheres were administered twice daily for three days. H. Pylori clearance was evaluated by assessing colony count.

Results: Treatment with plain drug solution (90 mg/kg amoxicillin and 45 mg/kg clarithromycin) resulted in a colony count of log 1.25 ± 0.56 CFU and clearance rate of 60 %, while mucoadhesive microspheres-loaded dose of 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin resulted in complete (100 %) eradication of H. pylori infection.

Conclusion: The developed mucoadhesive amoxicillin/clarithromycin microspheres can potentially be used to effectively eradicate H. pylori infection.

Keywords: Amoxicillin, Carbopol, Clarithromycin, H. pylori, Hydroxypropyl methylcellulose, Microspheres, Mucoadhesive

INTRODUCTION

Colonization of gastric mucosa by Helicobacter pylori is considered to be the cause of chronic gastritis, a strong risk factor for peptic ulcer disease and gastric cancer [1,2]. Amoxicillin trihydrate and clarithromycin combinations are the standard first-line H. pylori eradication treatment recommended by the American College of Gastroenterology (ACG) [3], the Maastricht III Consensus Conference [4], the Asia-Pacific Consensus Guidelines (APC) [5] and more recently, Maastricht IV/Florence Consensus Report [6].

Over the last decade, it has been widely reported that the success of H. pylori eradication treatment is falling [7]. Incomplete eradication of H. pylori is mainly due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where H. pylori exists [8,9].
Considering the above facts, we have recently formulated mucoadhesive microspheres of amoxicillin trihydrate and clarithromycin by using carbopol 974P, HPMC K4M and Eudragit RS 100 to achieve the mucoadhesive, extended/controlled release property and in vitro studies were carried out in order to select the best formulation [10-13]. H. pylori colonizes the stomach where the pH is acidic. In acidic pH, however, Carbopol 974P does not dissociate completely and also forms a less viscous gel, which significantly affect its release property. To optimize its extended/controlled release property and mucoadhesiveness, a combination of carbopol 974P and HPMC K4M was used to prepare mucoadhesive microspheres in the present study. The gelling nature of HPMC K4M is not affected by pH of the environment due to its non-ionic nature. Eudragit RS 100 was used as matrix polymer to disperse the mucoadhesive polymers and also because of its mucoadhesive property.

The objective of the present work was to prepare and evaluate in vivo H. pylori clearance efficacy of mucoadhesive microspheres of amoxicillin and clarithromycin with the aid of Eudragit RS, Carbopol 974P and HPMC [4-7].

**EXPERIMENTAL**

**Animals**

Six week-old male Wistar rats, weighing 180 to 220 g, were used to evaluate in vivo efficacy of the prepared microspheres. Amoxicillin trihydrate and clarithromycin were obtained as gifts from Macleods Pharmaceuticals Ltd, Mumbai, India. All the excipients were of pharmacopoeial grade and other chemicals used were of analytical grade.

**Preparation of culture**

Brucella chocolate agar media were used for in vivo studies [14]. Brucella chocolate agar media was prepared by sterilizing readymade media after dissolving in distilled water followed by addition of 10 – 20 % freshly collected sheep blood. Vancomycin (10 mg/L), amphoterecin B (4 mg/L), polymyxin B sulfate (2500 IU/L) and trimethoprim (5 mg/L) were added to this media. Human biopsy sample, which were collected from gastric ulcer patient with consent at Krishna hospital, Bhavani, India. The presence of H. pylori bacteria in biopsy was confirmed by modified Gram staining and positive urease tests [14]. The biopsy sample was swabbed on Brucella chocolate agar media culture plates. Plates were incubated in a candle jar at 37 °C under microaerophilic environment. Pad of cotton soaked with water were kept in the candle jar to maintain 90-100% humidity. The plates were examined after 72 hrs to see the colony growth. The organism was identified on the basis of modified Gram staining and urease tests. The colonies were found to be translucent and gray with size range of 0.5 -1 µm. The bacteria were then subcultured in Brucella broth medium.

**Induction of H. pylori infection in animals**

The in vivo study was performed in Wistar rats. The protocol of the experiment was approved by the Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Proposal no. 01PH10JAN10) and the International Guidelines for Handling of Laboratory Animals [15]. The number of bacteria was determined as 1 optical density (OD) unit corresponding to $10^9$ colony-forming unit (CFU)/mL bacteria at 600 nm in a Shimadzu UV/Visible spectrophotometer. H. pylori infection in animal model was established according to Qian’s method [16]. The wistar rats were deprived of food for 24 hrs before and 4 hrs after H. pylori inoculation, but were otherwise afforded free access to food and tap water. 0.3 mL of broth containing $10^5$ CFU/ml of H. pylori was inoculated into the stomach by intragastric gavage using a sterile gastric canula (6-week-old male Wistar rats, weight 180 to 220 grams). Then, the rats were fed for 4 weeks. The rats were kept with proper maintenance of temperature (20 to 25 °C), relative humidity (55%), and light/dark cycle (12 h/12 h). Four weeks after, the rats were used for in vivo studies.

**Therapy**

H. Pylori infectious animals were randomly divided in different groups (each group consists of 5 wistar rats) and different formulations were administered twice daily for three consecutive days as per the following dosing schedule.

The animals in group 1 were treated with sterile water (control group). Group 2 animals were treated with solution containing both drugs, dose equivalent to 22.5mg/kg amoxicillin and 11.25 mg/kg clarithromycin. Group 3 animals were treated with solution containing both drugs, dose equivalent to 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin. Group 4 animals were treated with a solution containing both drugs at a dose equivalent to 90 mg/kg amoxicillin and 45
mg/kg clarithromycin. Group 5 animals were treated with mucoadhesive microspheres, containing both drugs (mixture of amoxicillin trihydrate loaded and clarithromycin loaded) dose equivalent to 22.5 mg/kg amoxicillin and 11.25 mg/kg clarithromycin. Group 6 animals were treated with mucoadhesive microspheres, containing both drugs (mixture of amoxicillin trihydrate-loaded and clarithromycin-loaded) at a dose equivalent to 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin. Group 7 animals were treated with mucoadhesive microspheres, containing both drugs (mixture of amoxicillin trihydrate-loaded and clarithromycin-loaded) at a dose equivalent to 90 mg/kg amoxicillin and 45 mg/kg clarithromycin.

**H. pylori eradication confirmation**

One day after the administration of the final dose the rats were sacrificed with ether anesthesia and stomachs were excised. Stomach was homogenized in homogenizer with 3 mL of Brucella broth. Serial dilutions were made and aliquots (100 µL) of the dilutions were applied to Brucella agar plates. The plates were incubated at 37 °C under microaerophilic atmosphere for 72 hrs under the microaerophilic condition in a candle jar. The viable cell counts for each stomach were calculated by counting the number of colonies on agar plates [14]. The colonies were identified for *H. pylori* by morphology and urease test. The number of colonies per plates were counted and expressed as the log CFU per gastric wall.

**Statistical analysis**

The results were expressed as mean ± standard error (SE). Differences between the control and drug-loaded formulation treatment groups were statistically analyzed by ANOVA, followed by Dunnet’s multiple comparison test, as post-test (n = 5). Statistical significant differences between groups were defined as p < 0.05. Calculations were performed with Graph Pad Instat software (Graph Pad Software Inc, San Diego, USA).

**RESULTS**

*In vivo* clearance after multiple administrations of various formulations containing the combination of amoxicillin trihydrate and clarithromycin in under-fed conditions are presented in Table 1. The mean bacterial count (log colony forming units; log CFU) of the control group, which given only sterile water was log 8.55 ± 0.46 CFU. The mean bacterial count after oral administration of plain drugs decreased upon increasing the drug dose but complete eradication was not achieved even with the highest dose (90 mg/kg amoxicillin and 45 mg/kg clarithromycin).

Treatment with a plain drug solution in a dose of 22.5 mg/kg amoxicillin and 11.25 mg/kg clarithromycin gave a mean bacterial count of log 4.65 ± 0.93 CFU and clearance rate of 40 %. Treatment with plain drug solution in a dose of 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin gave a mean bacterial count of log 2.34 ± 0.88 CFU and clearance rate of 60 %. A higher plain drug dose (90 mg/kg amoxicillin and 45mg/kg clarithromycin) resulted in a colony count of log 1.25 ± 0.56 CFU and clearance rate of 60 %. Incomplete clearance of drug solutions might be due to the unavailability of 100% drug due short residence time of drugs in the stomach and the low concentration of the drugs reaching the bacteria under the gastric mucus layer.

Mean bacterial colony count after oral administration of microspheres containing dose equivalent to 22.5 mg/kg amoxicillin and 11.5 mg/kg clarithromycin was log 1.04 ± 0.29 CFU. In the case of microspheres containing dose equivalent to 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin and 90 mg/kg amoxicillin and 45mg/kg clarithromycin, bacterial colony was not detected. It means treatment with mucoadhesive microspheres loaded drug dose of 45 mg/kg amoxicillin and 22.5 mg/kg clarithromycin or higher resulted in complete eradication of *H. pylori* infection and thus the mucoadhesive microspheres were found to be effective in the treatment of *H. pylori* infections.

**DISCUSSION**

The rationale for developing and evaluating mucoadhesive microspheres was the need of a carrier system which could deliver the drug to the desired site of action in therapeutic concentrations over a prolonged period of time. In order to improve the efficacy of the anti *H. pylori* agents, localized delivery in the stomach was proposed. A combination approach, i.e., mucoadhesive and extended/controlled drug delivery system, was explored for the effective and improved treatment of *H. pylori* infection in the present study. Mucoadhesive drug delivery systems thus provide sustained drug release by localizing drug in the mucosal region where the bacteria reside [17]. This would also increase the antibiotic concentration in the luminal region of the stomach by promoting the diffusion of the antibiotics in the epithelial cell layer and thus would be effective in inhibiting the growth of bacteria in that region [8].

**Table 1**: Bacterial counts of various formulations on *H. pylori* under-fed condition

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<thead>
<tr>
<th>Formulation</th>
<th>Bacterial Count (log CFU)</th>
<th>Clearance Rate (%)</th>
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<tbody>
<tr>
<td>Control</td>
<td>8.55 ± 0.46</td>
<td>0</td>
</tr>
<tr>
<td>Plain Drug</td>
<td>4.65 ± 0.93</td>
<td>40</td>
</tr>
<tr>
<td>45 mg/kg</td>
<td>2.34 ± 0.88</td>
<td>60</td>
</tr>
<tr>
<td>90 mg/kg</td>
<td>1.25 ± 0.56</td>
<td>60</td>
</tr>
<tr>
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<td>1.04 ± 0.29</td>
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Up to 100 % \( H. \) pylori clearance rate was achieved. Mucoadhesive microspheres gave better and higher \( H. \) pylori eradication rates than plain drug solutions. Incomplete eradication of \( H. \) pylori might be the short residence time of the plain drugs in the gastric mucosa of animals and possible degradation of drugs in the acidic conditions in the stomach which was reported earlier \[8, 9, 17\]. This clearly confirms the hypothesis that encapsulation of drugs in the mucoadhesive microspheres is necessary to protect the drug in the acidic environment and also to increase their retention in the stomach for prolonged duration, which gives the highest efficacy in eradication of \( H. \) pylori infection.

It seems from the results of the in vivo bacterial clearance study that, unlike the plain drug, mucoadhesive microspheres are plug and seal the ulcer area, retain in the stomach for prolonged duration, release the drug slowly, and also preventing the entire drug from being exposed to the acidic media, thereby producing better \( H. \) pylori clearance. The use of the mucoadhesive microspheres can enhance stomach-specific delivery, increase gastric residence time, decrease diffusional distance, and allow more antibiotics to reach the infected site.

**CONCLUSION**

This study demonstrates that mucoadhesive microspheres of amoxicillin/clarithromycin, unlike the solution mixture of the plain drugs, is capable of achieving complete clearance of \( H. \) pylori infection and therefore would be suitable for the treatment of \( H. \) pylori infection.

**REFERENCES**


