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Development and evaluation of a novel floating in situ gelling system of amoxicillin for eradication of *Helicobacter pylori*

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Abstract

The aim of this study was to develop a new intra-gastric floating in situ gelling system for controlled delivery of amoxicillin for the treatment of peptic ulcer disease caused by *Helicobacter pylori* (*H. pylori*). Gellan based amoxicillin floating in situ gelling systems (AFIG) were prepared by dissolving varying concentrations of gellan gum in deionized water containing sodium citrate, to which varying concentrations of drug and calcium carbonate, as gas-forming agent, was added and dissolved by stirring. The formulation variables like concentration of gellan gum and calcium carbonate significantly affected the in vitro drug release from the prepared AFIG. The in vivo *H. pylori* clearance efficacy of prepared AFIG in reference to amoxicillin suspension following repeated oral administration to *H. pylori* infected Mongolian gerbils was examined by polymerase chain reaction (PCR) technique and by a microbial culture method. AFIG showed a significant anti-*H. pylori* effect in the in vivo gerbil model. It was noted that the required amount of amoxicillin for eradication of *H. pylori* was 10 times less in AFIG than from the corresponding amoxicillin suspension. The results further substantiated that the prepared AFIG has feasibility of forming rigid gels in the gastric environment and eradicated *H. pylori* from the gastrointestinal tract more effectively than amoxicillin suspension because of the prolonged gastrointestinal residence time of the formulation.

Keywords: Oral in situ gelling system; Controlled delivery; Amoxicillin; Gastric residence time; Floating drug delivery

1. Introduction

Helicobacter pylori (*H. pylori*) is one of the most common pathogenic bacterial infections, colonizing an estimated half of all humans (Marshall and Warren, 1984). It is associated with the development of serious gastro duodenal disease—including peptic ulcers, gastric lymphoma and acute chronic gastritis (Crescenzi et al., 1990). *H. pylori* reside mainly in the gastric mucosa or at the interface between the mucous layer and the epithelial cells of the antral region of the stomach (Peterson, 1991). The discovery of this microorganism has revolutionized the diagnosis and treatment of peptic ulcer disease. Most antibacterial agents have low minimum inhibitory concentrations (MIC) against *H. pylori* in culture. And also single antibiotic therapy is not effective for the eradication of *H. pylori* infection in vivo. This is because of the low concentration of the antibiotic reaching the bacteria under the mucosa, instability of the drug in the low pH of gastric fluid and short residence time of the antibiotic in the stomach (Shah et al., 1999). Combination of more than one antibiotic and anti-secretory agent are required for complete eradication of *H. pylori* but these regimens are not fully effective. Patient compliance, side effects and bacterial resistance are the other problems. Other than the multi-antibiotic therapy, different therapeutic strategies have been examined to completely eradicate *H. pylori* from the stomach.

One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach (Yokel et al., 1995; Shah et al., 1999). Better stability and longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori* (Umamaheshwari et al., 2004).

The reason for the incomplete eradication of *H. pylori* is probably due to short residence time of antimicrobial agents in the

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stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists (Cooreman et al., 1993; Atherton et al., 1995). The other reason may be the degradation of antibiotics in gastric acid (Axon, 1994; Giacomo et al., 2001). Therefore, some researchers had prepared and reported new amoxicillin formulations, such as floating tablets, mucoadhesive tablets, pH sensitive excipients composition mucoadhesive microspheres, etc., which were able to reside in stomach for an extended period for more effective *H. pylori* eradication (Umamaheshwari et al., 2003; Hilton and Deasy, 1992). 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. As 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. Therefore, it is necessary to design drug delivery systems that not 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) (Katayama et al., 1999).

Keeping above facts in mind we made an attempt to develop a new floating in situ gelling system of amoxicillin using gellan as gelling polymer with a potential to use in treatment of *H. pylori* caused stomach ulcer. The proposed new gellan based amoxicillin floating in situ gelling systems (AFIG), would have the advantage of ease of administration, as being a liquid, and also be more patient compliant.

Amoxicillin is a semisynthetic, orally absorbed, broad-spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent (Suleymanlar et al., 1999; Vakil and Cutler, 1999). Gellan gun is a bacterial anionic deacetylated polysaccharide secreted by *Pseudomonas elodea*. It has a characteristic gelling property, which is temperature and ionic dependant (Miyazaki et al., 1999, 2001). The basic strategy adopted in this study involved incorporation of calcium carbonate and sodium citrate in a gellan gum-amoxicillin dispersion. Initially, the calcium carbonate becomes soluble in the acidic environment of the stomach, and the released calcium ions then are complexed by the sodium citrate. However, a slow conversion of the complexed calcium into free calcium causes gelation of gellan, the gelled material floats upwards in the stomach, with a potential to release its drug over a period of time. The calcium carbonate present in the formulation, releases carbon dioxide in the gastric environment, thereby making the formulation buoyant, thus prolonging the residence time.

2. Materials and methods

2.1. Materials

Amoxicillin was gifted by Ranbaxy Laboratories Ltd. (New Delhi, India) and Gellan gum (Gelrite®) was purchased from CP Kelco Company (Santiago, California, USA). Modified Skirrow's medium, Brucella broth and fetal calf serum (FCS) were purchased from Himedia (Mumbai, India). Agarose was purchased from FMC BioProducts (Rockland, USA) and Taq DNA polymerase was purchased from Takara Shuzo, Otsu, Shiga, Japan. All other reagents were of analytical grade.

2.2. Animals

Six-week-old male specific pathogen free Mongolian gerbils (body weight 50–60 g) were purchased from Central Drug Research Institute (Lucknow, India) and were maintained under standard laboratory conditions (room temperature, $23 \pm 2\,^{\circ}C$; relative humidity, $55 \pm 5\%$; 12-h light:12-h dark cycle) with free access to a commercial rodent diet and tap water.

3. Methods

3.1. Preparation of in situ gelling solution

Gellan gum, at solution concentrations of 0.25–1.0% (w/v) were prepared in deionized water containing sodium citrate (0.25%, w/v). Low level of cations present in the solution was sufficient to hold the molecular chains together and inhibit hydration. The gellan gum solutions were heated to 90 °C with stirring. After cooling below 40 °C, various concentrations of calcium carbonate and drug were added and dispersed well with continuous stirring .The resulting gellan in situ gel solution containing amoxicillin was finally stored in amber colour narrow mouth bottles until further use.

3.2. Measurement of viscosity of in situ gelling solutions

The viscosity of sols were determined by cone and plate viscometer with cone angle 1° 34 (TV-20H, model E. Tokimec Co., Tokyo, Japan) at 5 or 20 °C using 1 ml aliquot of sample. Viscosity measurement for each sample was done in triplicate, with each measurement taking approximately 30 s.

3.3. In vitro gelation study

The gelation studies were carried out as described previously (Zhidong et al., 2006) with slight modification. The gelation cells were fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (simulated gastric fluid (SGF) of pH 1.2, without enzymes). Within the cells located at the bottom was a 250 μl transparent plastic cup to hold the gel sample in place after its formation. Then, 100 μl of the preparation was carefully placed into the cavity of the cup using micropipette, and 2 ml of the gelation solution (SGF) was added slowly in reservoir. Gelation was observed by visual examination.

3.4. In vitro floating study

The in vitro floating study was determined using USP dissolution apparatus II having 500 ml of simulated gastric fluid (pH