International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 2, 2011

Research Article

FORMULATION OF CLARITHROMYCIN LOADED MUCOADHESIVE MICROSPHERES BY EMULSIFICATION-INTERNAL GELATION TECHNIQUE FOR ANTI- *HELICOBACTER PYLORI* THERAPY

SAMBATHKUMAR. R*, VENKATESWARAMURTHY. N, VIJAYABASKARAN. M, PERUMAL .P

Department of Pharmaceutics, J. K. K. Nataraja College of Pharmacy, Komarapalayam - 638 183, India,
Email: sambathju2002@yahoo.co.in

Received: 28 Nov 2010, Revised and Accepted: 28 Dec 2010

ABSTRACT

Helicobacter pylori (H. pylori) infection has a strong association with chronic active gastritis and duodenal ulcer (DU). *H. pylori* infection can be detected in 90% of patients with DU and 70% of those with gastric ulcers. The most successful and universal treatment is triple therapy, three drugs twice a day for 1 week (proton pump inhibitor [PPI], amoxicillin and clarithromycin). However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation. One of the reason for incomplete eradication is probably that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists. One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach. Considering above issue, we have prepared clarithromycin loaded microspheres for anti – *H.pylori* Therapy. The mucoadhesive microspheres prepared by using sodium alginate alone and in combination with HPMC K4M and carbopol 974 P. Clarithromycin loaded mucoadhesive microspheres were successfully prepared by emulsification-internal gelation technique with a maximum incorporation efficiency of 93 %. The scanning electron microscopic study indicated that the microspheres were spherical in shape. The in vitro wash-off test indicated that the microspheres had good mucoadhesive properties. *In vitro* were conducted in 0.1 N HCL. The preliminary results show great promise for this delivery strategy in the treatment of *H. Pylori* infection.

INTRODUCTION

Helicobacter pylori (H. pylori) infection has a strong association with chronic active gastritis and duodenal ulcer (DU). Ingestion of *H. pylori* has been shown to produce acute antral gastritis. [1-3] *H. pylori* infection can be detected in 90% of patients with DU and 70% of those with gastric ulcers. The most successful and universal treatment is triple therapy, three drugs twice a day for 1 week (proton pump inhibitor (PPI), amoxicillin and Clarithromycin.

However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation[4]. One of the reason for incomplete eradication is probably that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists. One way to improve the efficacy in eradicating the infection is to deliver the antibiotic locally in the stomach[5,6]. Longer residence time will allow more of the antibiotic to penetrate through the gastric mucus layer to act on *H. pylori*.

Mucoadhesive microspheres include microparticles and microcapsules (having a core of the drug) of 1-1000 µm in diameter and consist either entirely of a mucoadhesive polymer or having an outer coating of it, respectively. Microspheres, in general, have the potential to be used for targeted and controlled release drug delivery, but coupling of mucoadhesive properties to microspheres has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs due to a high surface to volume ratio, a much more intimate contact with the mucus layer, specific targeting of drugs to the absorption site. Mucoadhesive microspheres can be tailored to adhere to any mucosal tissue including those found in stomach, thus offering the possibilities of localized as well as systemic controlled release of drugs. The application of mucoadhesive microspheres to the mucosal tissues of gastric epithelium is used for administration of drugs for localized action. Mucoadhesive microspheres are widely used because they release the drug for prolong period, reduce frequency of drug administration and improve the patient compliance[7].

Considering above advantages, we have prepared Clarithromycin loaded microspheres for anti –*H.pylori* Therapy. The mucoadhesive microspheres prepared by using sodium alginate alone and in combination with HPMC k4M and carbopol 974 P.

MATERIALS AND METHODS

Materials

Clarithromycin was supplied as a gift sample by M/s Zydus Cadila Health Care Ltd. (Ahmedabad, India). sodium alginate, Hydroxyl propylmethyl cellulose K4M (HPMC K4M) and carbopol 974P (Loba Chemie Pvt. Ltd., Mumbai), barium carbonate, chloroform, hydrochloric acid and glacial acetic acid (Ranbaxy Fine Chemicals, Chandigarh), heavy liquid paraffin and Span 80 (S.D. Fine chemicals, Mumbai), sodium hydroxide pellets (Qualigens Fine Chemicals, New Delhi), potassium dihydrogen phosphate (S.D. Fine chemicals, Mumbai). All the solvent and chemicals used were of analytical grade satisfy ing pharmacopoeial standards.

Preparation of microspheres

Microspheres containing clarithromycin were prepared employing sodium alginate alone and in combination with HPMC K4M and carbopol 974P. The homogeneous polymer(s) solution was prepared in distilled water stirred magnetically with gentle heat. The drug and cross-linking agent were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with a needle of size no. 23 into light liquid paraffin containing 1.5% span 80 and 0.2% glacial acetic acid being kept under magnetic stirring at 100 rpm. The microspheres were retained in the light liquid paraffin for 30 min to produce rigid discrete particles. They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil. The microspheres were dried at 40^0 under vacuum for 12 h. The compositions of the microspheres formulations are listed in Table 1.

Table 1: Formulation composition of mucoadhesive microspheres of Clarithromycin

Formulation code	Sodium Alginate (%)	HPMC* K4M (%)	carbopol 974P (%)
F1	1.5	-	-
F2	2.5	-	-
F3	3.5	-	-
F4	1.5	2	-
F5	2.5	2	-
F6	3.5	2	-
F7	1.5	-	1
F8	2.5	-	1
F9	3.5	-	1

* HPMC = Hydroxypropyl methyl cellulose

Table 2: Physico-chemical characteristics of the Clarithromycin loaded mucoadhesive microspheres

Formulation code	Incorporation efficiency (%)± SD	Particle size (mean ± SD) µm	Mucoadhesion (%)± SD
F1	66±1.88	602±1.03	65± 2.02
F2	84±2.03	650±2.18	68±2.77
F3	86±1.99	770±2.47	74± 2.63
F4	84±2.09	708± 3.53	81±1.34
F5	85±2.88	740±2.12	83±1.03
F6	93 ±2.02	784± 5.11	87±1.11
F7	71±1.66	650± 3.03	85±2.05
F8	73±1.03	680±2.04	88±2.33
F9	90±2.04	713±1.88	93±1.66

Degradation of Clarithromycin in pH 1.2[8]

Clarithromycin was reported to be unstable [9,10] in mediums with low pH. Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from the microspheres. Hence, inorder to calculate correct amount of the drug released the degradation rate constant will need to be determined. The degradation rate of the antimicrobial agent at pH 1.2 was examined by reported method with slight modification. A known amount of clarithromycin was added to the medium, which was preheated at 37ºC±0.2°C, to make a final concentration of 10.0 µg/ml. An aliquot of the medium was withdrawn at predetermined time intervals and neutralized with a NaOH solution before being quantified by HPLC. Then the solution was filtrated through a 0.45 µm syringe filter then analyzed for clarithromycin content by reversed-phase high performance liquid chromatography (RPHPLC) method using a mobile phase consisting of acetonitrile–aqueous 0.05 M phosphate buffer solution of pH 4.0 (40:60 v/v). The apparatus used for HPLC analysis was an Agilent 1100 quaternary pump, with a variable wavelength detector, thermostatted autosampler and column thermostat. A Hypersil ODS C18 column (250mm×4.6mm ID, 5 µm, Thermo, UK) was fitted with a Phenomenex guard column packed with octadecyl C18 (Phenomenex, USA).The column temperature was maintained at 40°C and flow rate of 1ml/min. The The concentrations of the parent drug remaining were analyzed by RP-HPLC assay. The degradation of clarithromycin was assumed to follow pseudo-first order kinetics, which is described by the following equation:

$$C=C_o e^{-kt}$$

in which C is the concentration of clarithromycin remaining at time t, C_o is the initial concentration of clarithromycin, and k is the pseudo-first order degradation rate constant. The half-life ($t_{1/2}$) of clarithromycin was determined from the pseudo-first order degradation rate constant. Degradation rate constant used to correct the drug release data obtained in acidic media.

Incorporation efficiency

The amount of clarithromycin present in the microspheres was determined by extracting the drug into phosphate buffer of pH 7.4 under magnetic stirring for a period of 2 h. The solution was filtered through Whatman filter paper no.5, suitably diluted. clarithromycin content estimated by by high performance liquid chromatography and the conditions for the HPLC assay were the same as before. The incorporation efficiency was calculated by the following formula: Incorporation efficiency (%) = Experimental drug content × 100 /Theoretical drug content

In vitro evaluation of mucoadhesivenes[11]

A strip of goat intestinal mucosa was mounted on a glass slide and accurately weighed mucoadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45^0. 0.1 N HCL (pH 1.2), previously warmed to 37 ± 0.5^0C, was circulated to the cell over the microspheres and membrane at the rate of 1 ml/min with the help of pump. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50^0C. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated by

$$\text{Percentage mucoadhesion} = \frac{Wa - Wl \times 100}{Wa}$$

Where Wa = weight of microspheres applied; W1 = weight of microspheres leached out.

Size distribution of microspheres

The prepared microspheres were sized by using a Malvern 2600 Laser Diffraction Spectrometer. The size of the microspheres was determined in n-hexane as a non-dissolving dispersion medium and the particles were suspended mechanically by magnetic stirring during the measurement.

Scanning electron microscopy (SEM)

Scanning electron photomicrograph of amoxicillin loaded mucoadhesive microspheres were taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch F6 is depicted in Fig. 1.

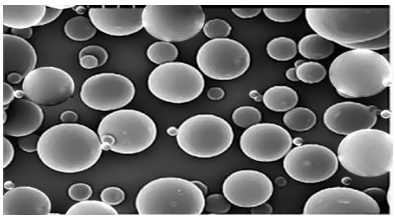

Fig. 1: SEM photograph of Clarithromycin loaded mucoadhesive microspheres

In vitro drug release studies

Release of clarithromycin from the microspheres was studied in 0.1N HCL (900 mL) using a USP XXIII paddle method Dissolution Rate Test Apparatus (Dissco 2000, Labindia) with a rotating paddle stirrer at 50 rpm and 37° ± 1°C. A sample of microspheres equivalent to 25 mg of clarithromycin was used in each test. Samples of dissolution fluid were withdrawn through a filter (0.45 µm) at different time intervals and were assayed for drug release by high performance Liquid chromatography. The drug release experiments were conducted in triplicate (n = 3).

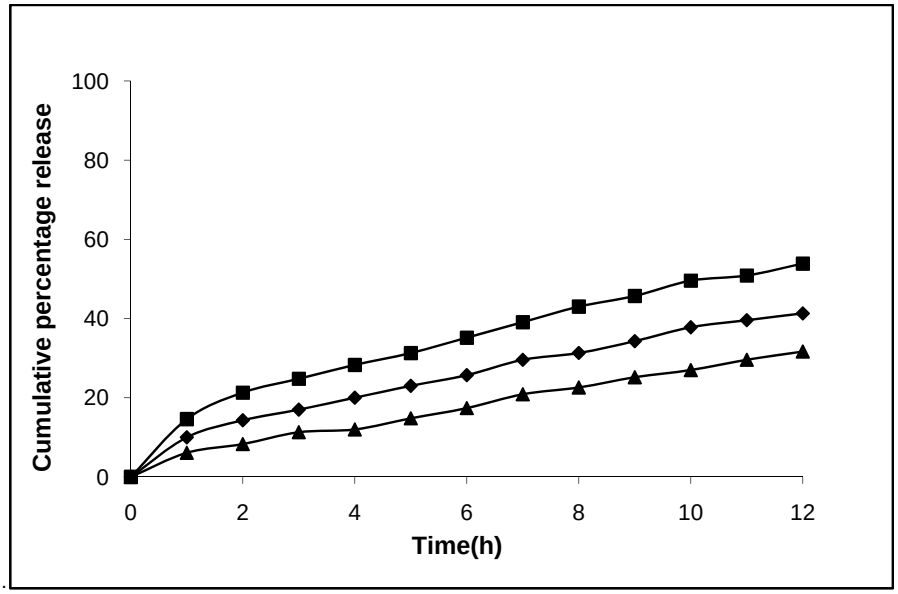

Fig. 2: Effect of concentration of Sodium alginate on drug release pattern of microspheres in 0.1 N HCl, (pH 1.2) for the formulations F1 ( ), F2 ( ) and F3 ( ).

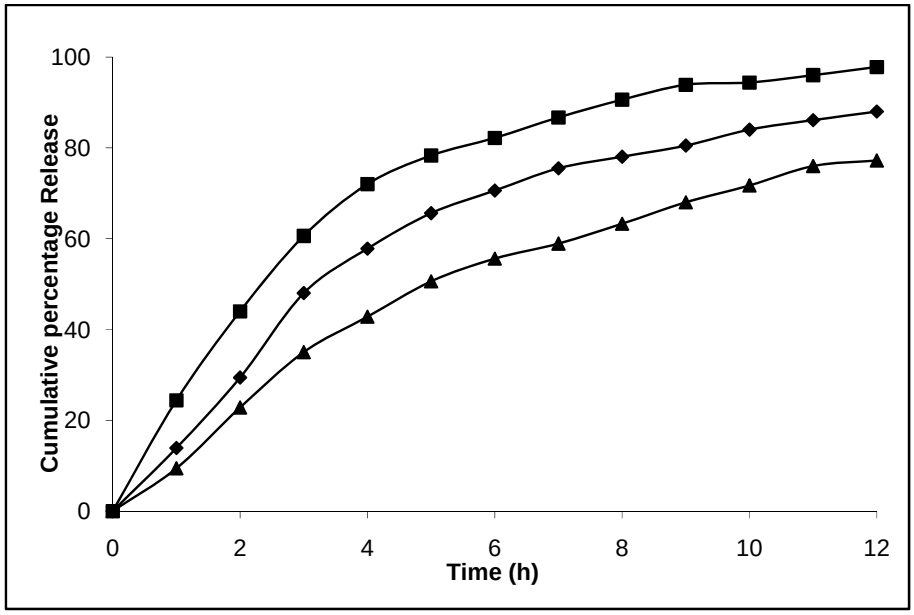

Fig. 3: Effect of concentration of HPMC K4M on drug release pattern of microspheres in 0.1 N HCl, (pH 1.2) for the formulations F4( ),F5( ) and F6( ).

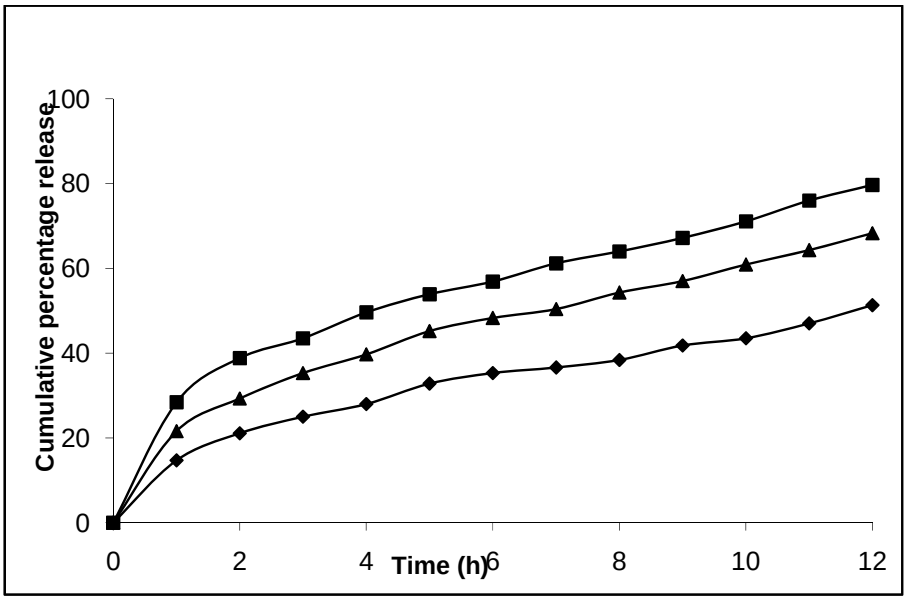

Fig. 4: Effect of concentration of Carbopol on drug release pattern of microspheres in 0.1 N HCl, (pH 1.2) for the formulations F7 ( ), F8 ( ) and F9( ).

RESULTS AND DISCUSSION

Clarithromycin loaded mucoadhesive microspheres were prepared by emulsification-internal gelation technique. The emulsification-internal gelation technique use an oil soluble acid (0.2% glacial acetic acid) in the external oil phase, which diffuse through the oil-water interface into the polymeric dispersed globules containing barium carbonate, resulting in the release of free Ba^{2+}. The sodium ion (Na^{+}) of alginate is exchanged with Ba^{2+}initiating gelation reaction to form barium alginate gel beads. The resulting microspheres were found to be discrete, spherical, free flowing and of the monolithic matrix type. The microspheres were uniform in size with a mean size range of 602±1.03to 784± 0.03 µm. The size of the microspheres was in increasing trend with increasing the alginate concentration. This may be due to the increase in viscosity, which in turn increases in droplet size during addition of the polymer dispersion to the harvesting medium. The effects of alginate concentrations and polymer compositions on the drug incorporation efficiency of microspheres are shown in Table 2. The highest incorporation efficiency (96±2.02%) was achieved with 3.5% w/v sodium alginate in combination with 2% HPMC. Three diferent concentrations of sodium alginate (1.5%,2.5% and 3.5%) were used. The higher incorporation efficiency was observed as the concentration of alginate increased. This may be attributed to the greater availability of active barium binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of sodium alginate increased, resulting in the formation of nonporous microspheres. The drug loading efficiency greatly improved when alginate was blended with carbopol 974 P at 1% level.

In vitro drug release studies were carried out in the simulated gastric fluid (0.1 N HCl). Sodium alginate at three different concentrations (1.5%,2.5% and 3.5%) alone and in combination with 1% w/v of HPMC K4M and/or carbopol 934 P was utilized for the preparation of microspheres. It was observed that the amount of drug release decrease with an increase in the concentration of sodium alginate. It can be attributed to an increase in the densities of the polymer matrix resulting in larger microspheres and this in turn increase the diffusion path length, which the drug molecules have to be traverse.

To optimize drug release and mucoadhesiveness of the microspheres, HPMC K4M and carbopol 974P were blended with the alginate matrix. sodium alginate widely used as sustained release matrix because of its insoluble and low permeability in acidic pH. carbopol 974P and HPMC K4M incorporated in matrix to create hydrophilic passage inside the microsphere to help the drug release. carbopol forms gel when exposed to a pH environment above 4-6. Lots of carboxyl groups of carbopol ionize at pH 3.6, resulting in repulsion between the anions and further increasing the swelling of the polymer, while the carbopol groups will not ionize at pH 1.3[12]. Due to this nature it minimizes passage of dissolution medium inside the microsphere compared to HPMC. But Microspheres prepared with blends of sodium alginate and carbopol 974 showed highest mucoadhesiveness. While considering mucoadhesiveness and sustained release property, microspheres prepared with blends of sodium alginate and carbopol 974 may be suitable for *H. pylori* eradication.

CONCLUSION

H. pylori colonize the gastric mucosa leading to gastritis, gastric ulcer, and gastric carcinoma. To increase the efficacy of eradicating the infection, a localized delivery system of anti-*H. pylori* agents in the stomach is required. Clarithromycin microsphere formulation was prepared to increase the local concentration of the antibiotic in the stomach and, thus eradicate *H. pylori* infection. *In vitro* studies clearly indicates that the prepared formulations possess good bioadhesive properties. These properties enable the microspheres to adhere to the gastric mucosal surface and stay in stomach for prolonged periods and could ensure the stability of amoxicillin in gastric environment, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms. Further studies are planned to examine the gastric residence time of the microsphere formulation and the efficacy in eradicating *H. pylori* infection in suitable animal model.

REFERENCES

1. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfill Koch's postulates for pyloric Campylobacter. Med J Aust 1985;142:436-9.
2. Morris A, Nicholson G. Ingestion of Campylobacter pyloridis causes gastritis and raised fasting gastric pH. Am J Gastroenterol 1987;82:192-9.
3. Graham Y, Alpert LC, Smith JL, Yoshimura HH. Iatrogenic Campylobacter pylori infection as a cause of epidemic achlorhydria. Am J Gastroenterol 1988;83:974-80.

4. Bytzer P, O'Morain C. Treatment of Helicobacter pylori, Helicobacter,2010;10:40–46.
5. Yokel RA, Dicke KM, Goldberg AH. Selective adherence of a sucralfate-tetracycline complex to gastric ulcers: implications for the treatment of Helicobacter pylori. Biopharm Drug Dispos 1995; 16: 475–479.
6. Shah S, Qaqish R, Patel V, Amiji M. Evaluation of the factors influencing stomach-specific delivery of antibacterial agents for Helicobacter pylori infection. J Pharm Pharmacol. 1999;51: 667–672.
7. Vasir AK, Tambwekar K and Garg S. Bioadhesive microspheres as a controlled drug delivery system. Intl J Pharm 2003;14:13-32.
8. Zhepeng L, Weiyue L, Lisheng Q, Xuhui Z. In vitro and in vivo studies on mucoadhesive microspheres of amoxicillin. J Control Rel 2004;81:327-34.
9. Erah PO, Goddard AF, Barrett D, Shaw PN, Spiller RC, The stability of amoxycillin, clarithromycin and metronidazole in gastric juice:relevance to the treatment of Helicobacter pylori infection, J Antimicrob Chemother. 1997;39:5–12.
10. Nakagawa Y, Itai S, Yoshida T, Nagai T. Physicochemical properties and stability in the acidic solution of a new macrolide antibiotic, clarithromycin, in comparison with erythromycin, Chem Pharm Bull 1992;40:725–728.
11. Jain SK, Chourasia MK, Jain AK. Jain RK. Development and characterization of Mucoadhesive Microspheres Bearing Salbutamol for Nasal Delivery, Drug Deliv 2004;11:113–22.
12. Tao Y, Lu Y, Sun Y, Gu B, Lu W, Pan J. Development of mucoadhesive microspheres of acyclovir with enhanced bioavailability. Int J Pharm 2009;3:30-36.