RESEARCH ARTICLE

Development of Gastroretentive Mucoadhesive Solid Dosages Form Containing Amoxicillin Trihydrate and Ranitidine HCL for the Treatment of *Helicobacter pylori* Infections

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Abstract

Mucoadhesion can be explored in the design of a drug delivery system. Mucoadhesive drug delivery systems interact with the mucus layer covering the mucosal epithelial surface and mucin molecules, thus increasing the residence time of the dosage form at the site of absorption. The drugs having local action or possessing maximum absorption in the gastrointestinal tract (GIT) require an increased duration of stay in GIT. Gastro retentive mucoadhesive solid dosages form containing amoxicillin trihydrate and ranitidine HCL were formulated to prolong the residence time of the dosage form at the site of application or absorption, and it results in intimate contact of the dosage form with the underline absorption surface. FDA approves this combination for the treatment of *Helicobacter pylori* Infections. The formulation results in efficient absorption, enhanced bioavailability of the drugs due to a high surface-to-volume ratio and an improved therapeutic performance of the drug. The prolonged drug release resulted in reduced drug administration frequency and improved patient compliance. The optimized formulation was evaluated for various parameters i.e. hardness, friability, thickness, weight variation, drug content, dissolution study and swelling index.

Keywords: Gastric Retention Formulations (GRFs), Mucoadhesive, Amoxicillin Trihydrate, Ranitidine HCL, *Helicobacter pylori*

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Introduction

Oral route is undoubtedly most favored route of administration, but hepatic first-pass metabolism, drug degradation during absorption, mucus covering GI epithelia, and high mucus turnover are serious concerns of oral route. In recent years, gastrointestinal tract (GIT) delivery emerged as a most important route of administration. Bioadhesive retentive system involves the use of bioadhesive polymers, which can adhere to the epithelial surface in the GIT. Using bioadhesives would be achieved increase GI transit time and increase in bioavailability. Mucus is a translucent and viscid secretion which forms a thin, continuous gel blanket adherent to the mucosal epithelial surface. The mean thickness of this layer varies from about 50 to 450 µm in humans. It is secreted by the goblet cells lining the epithelia or by special exocrine glands with mucus cells acini. The exact composition of the mucus layer varies substantially, depending on the species, the anatomical location and pathological states. However, it has general composition.

Due to their considerable therapeutic advantages, oral controlled release (CR) dosage forms (DFs) have been developed for the past three decades. However, this approach has not been suitable for a variety of important drugs, characterized by a narrow absorption window in the upper part of the gastrointestinal tract, i.e. stomach and small intestine.

Mucoadhesive tablets, in general, have the potential to be used for controlled release drug delivery, but the coupling of mucoadhesive properties to tablet 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. Mucoadhesive tablets can be tailored to adhere to any mucosal tissue, including those found in stomach, thus offering the possibilities of localized and systemic controlled drug release. The application of mucoadhesive tablets to the mucosal tissues of gastric epithelium is used for the administration of drugs for localized action. Mucoadhesive tablets are widely used because they release the drug for prolong period, reduce frequency of drug administration and improve patient compliance.

Ranitidine and amoxicillin is one of the best combinations

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of solid dosages form used for treating *Helicobacter pylori* infection. *H. pylori* is very sensitive to amoxicillin both *in-vivo* and *in-vitro*. Amoxicillin works by inhibition of bacterial cell wall synthesis, leading to cell death. Amoxicillin has topical intraluminal activity at the level of the gastric mucosa, as well as systemic activity. The advantage of amoxicillin is that *H. pylori* does not develop resistance to it, and it can be used again in another antibiotic regimen. Ranitidine is a histamine 2-receptor-antagonist (H2RA). Ranitidine works by reducing the amount of acid your stomach produces. The agent has been developed for the treatment of patients with duodenal ulcer with or without infection with *H. pylori*. Combining Ranitidine HCL with amoxicillin trihydrate has resulted in an eradication rate of 72 to 80% compared to th The food and drug administration recently approved this combination. As mentioned earlier, the combination of Ranitidine HCL with amoxicillin trihydrate has been shown to be effective in eradicating *H. pylori* infection. Therefore, we have planned in the development of gastro retentive mucoadhesive tablet containing Amoxicillin Trihydrate and Ranitidine HCL which may result in improved patient compliance, easy to administration, increased residence time resulting in enhanced absorption and increased therapeutic efficacy of the drug with reducing frequency of drug administration.

Material and Methods

The chemicals used were of laboratory reagents grade and were used as they were procured. Amoxicillin trihydrate and ranitidine hydrochloride were procured from Sun Pharmaceuticals Industries Limited, Indore. Rest of the chemicals, Guar Gum (GG), Micro Crystalline Cellulose (MCC), polyvinyl pyrollidone K-30 (PVP), Talc and magnesium stearate wee procured from Central Drug House Pvt. Ltd. The distilled water was used in all experiments.

Instruments used in the studies were UV Spectrophotometer Shimadzu 1800, FT-IR, Electronic Weighing balance, Hardness tester, Friability test apparatus and Standard USP or IP dissolution apparatus.

Procedure

Preformulation study

Pre formulation study of both drug i.e. amoxicillin trihydrate and ranitidine HCL, are performed its include solubility, melting point, FTIR, Angle of repose, Bult and Tap density.

Calibration curves of both drugs are performed by UV visible spectrophotometer.

Calibration curve data of Ranitidine HCL

Preparation of standard stock solution

An accurately weighed quantity of ranitidine (10 mg) was dissolved in 100 mL of water to prepare a stock solution of 100 µg/mL of ranitidine.

Determination of absorption maxima (λ max)

Standard solution containing 10 µg/mL of ranitidine was scanned for the absorption maxima between 200 to 400 nm. Two absorption maxima were obtained i.e. one at 315 nm and the other at 231 nm.

Procedure

For the above stock solution, aliquots of 0.2,0.4 1.8,2.0 mL were withdrawn in a series of 10 mL volumetric flasks and diluted to 10 mL with water. This gave solution in a final concentration range of 2 to 20 µg/ mL. The absorbance of different photometers at λ max 315 nm. The data were processed using a computer and various parameters were obtained

Calibration curve data of Amoxicillin Trihydrate

Preparation of standard stock solution

An accurately weighed quantity of amoxicillin (10 mg) was dissolved in 100 mL of ethanol to prepare a stock solution of 100 µg/mL of amoxicillin.

Determination of absorption maxima (λ_{max})

Standard solution containing 10 µg/mL of amoxicillin was scanned for the absorption maxima between 200 to 400 nm. Two absorption maxima were obtained i.e. one at 228 nm and the other at 331 nm.

Procedure

For the above stock solution, aliquots of 0.2,0.4 1.8,2.0 mL were withdrawn in a series of 10 mL volumetric flasks and diluted to 10 mL with ethanol. This gave a solution in a final concentration range of 2-20 µg/mL. The absorbance of different photometers at λ_{max} 228 nm. The data were processed using a computer and various parameters were obtained.

Formulation of Mucoadhesive Tablets

Wet Granulation Method: Mucoadhesive tablets of ranitidine and amoxicillin were prepared by wet granulation technique using different concentrations of Guar gum and microcrystalline cellulose. All the ingredients were passed through sieve no. 85# and were mixed uniformly. Granulation was carried out with sufficient quantity of binder solution (PVP k30- 5% in water). Wet mass passed through sieve no. 12# and dried at 45 to 55°C for 1-hour. Dried granules were sized by sieve no. 18# and add magnesium stearate and talc. Then tablet punching machine was used to compress tablets containing 150 mg ranitidine and 250 mg amoxicillin at an average weight of 800 mg per tablet.

Post-compression parameters of mucoadhesive tablet:

Appearance

Twenty tablets of each formulation were taken to check any discoloration or surface roughness in the tablet formulation.

Weight variation test

To study weight variation tablets were weighed using an electronic balance and the test was performed according to

the Indian Pharmacopoeia.

Hardness

The hardness of five tablets was determined using the Monsanto hardness tester and the average values were calculated.

Thickness

The Thickness of the tablets was determined dosing manual Vernier Caliper. Five tablets were used, and average values were reported.

Friability

The friability of 20 tablets was measured by Roche friability by rotating for 4 minutes at 25 rpm upto 100 revolutions. Accurately weighed 20 tablets were placed into Roche friability for 100 revolutions then dedusted and weighed again.

$$\% \text{ Friability} = W0 - W/W0 \times 100$$

W0= Initial weight of tablet
W= Weight of tablet after revolution

Procedure to study Drug release of different formulation

Dissolution Time

In-vitro, release study Standard USP or IP dissolution apparatus have been used to study *in-vitro* release profile using both basket and rotating paddle. *In-vitro* release rate study of the mucoadhesive tablet of was carried out using the Apparatus 2 (Basket apparatus) method. Place the tablet in a dry basket at the beginning of each test. Lower the Basket before rotation operates the apparatus immediately at 50 rpm. Medium used for release rate study was 900 mL 0.1 N HCl during the course of study whole assembly was maintained at 37+0.5C. Withdraw a 5 mL of sample at specific time interval and replaced with 5 mL of fresh dissolution medium. The withdrawn samples were diluted with dissolution medium and then filtered with whatt'sman filter paper and assayed.

Procedure to study Swelling index of different formulation

Swelling Studies

The degree of swelling of mucoadhesive polymer is an important factor affecting adhesion. For conducting the study, a tablet was weighed and placed in a petridish containing 5 mL of 0.1 N HCl buffer pH 1.2 in 6 hours at regular intervals of time (1, 2, 4, and 6 h), the tablet was taken carefully by using filter paper. The swelling index was calculated using the following formula.

$$\text{Swelling Index (S.I)} = (Wt\text{-}Wo)/Wo \times 100$$

Where S.I = swelling index,

Table 1: Composition of mucoadhesive tablet

Composition	*Formulations*				
Amoxicillin trihydrate	F1 250	F2 250	F3 250	F4 250	F5 250
Ranitidine HCL	150	150	150	150	150
GG	150	50	100	125	150
MCC	150	250	200	175	90
PVP	50	50	50	50	210
Talc magnesium stearate	25 25	25 25	25 25	25 25	50 25
Total weight (mg)	800	800	800	800	800

Table 2: Pre-compression parameters of amoxicillin trihydrate

Formulation	*Bulk Density (g/mL)*	*Tapped Density (g/mL)*	*Angle of Repose*	*% Carr's Index*	*Hausner's Ratio*
F1	0.457	0.573	27.14	18.6	1.23
F2	0.521	0.577	27.30	17.2	1.19
F3	0.447	0.580	25.28	14.1	1.13
F4	0.459	0.564	27.01	18.2	1.18
F5	0.468	0.529	29.24	16.3	1.22

Table 3: Pre-compression parameters of ranitidine HCL

Formulation	*Bulk Density (g/mL)*	*Tapped Density (g/mL)*	*Angle of Repose*	*% Carr's Index*	*Hausner's Ratio*
F1	0.486	0.568	27.28	19.7	1.28
F2	0.459	0.560	27.51	18.7	1.24
F3	0.473	0.589	25.99	15.3	1.15
F4	0.399	0.615	27.84	17.8	1.18
F5	0.479	0.559	28.47	19.2	1.19

Table 4: Evaluation of physical parameters of different mucoadhesive tablets

Formulation	*Thickness (mm)*	*Hardness (kg/cm^2)*	*Friability (%)*	*Weight variation*
F1	3.1 ± 0.2	5.8 ± 0.19	0.65 ± 0.03	3.96 ± 1.28
F2	2.9 ± 0.3	6.0 ± 0.11	0.56 ± 0.09	4.39 ± 2.12
F3	3.2 ± 0.1	5.9 ± 0.12	0.59 ± 0.01	5.48 ± 1.11
F4	2.9 ± 0.5	5.6 ± 0.13	0.61 ± 0.02	3.92 ± 2.21
F5	3.0 ± 0.2	5.5 ± 0.21	0.58 ± 004	4.14 ± 1.21

Wt = weight of tablet a filter swollen at time t,
Wo = weight of the initial tablet

Results and Discussions

Mucoadhesive tablets of ranitidine and amoxicillin were prepared by wet granulation technique using different

concentration of Guar gum and micro crystalline cellulose. Five formulation from F1 to F5 are prepared, total weight of tablet is 800 mg. (Table 1).

Evaluation of Mucoadhesive Tablet

Evaluation of mucoadhesive table are performed by different parameters like % drug content, % water absorption, mucoadhesive strength. (Table 5)

%water absorpt ion studies done by Agar at 5% w/v was dissolved in hot water and then transferred to a petri dish and was allowed to be solidified. Prior to the study, six tablets were placed in a vacuum overnight to remove moisture. They were weighed initially and then positioned on the top of the agar and incubated at 37 °C for one hour. At the end of the test, the tablets were reweighed and the percent moisture absorption was calculated using the formula:

$$\% \text{ Moisture Absorption} = \frac{Wi \times Wf}{Wi} \times 100$$

Where Wf is the final weight and Wi is the initial weight of the tablets

Mucoadhesive/bioadhesive strength

A modified physical balance was used to measure the strength of mucoadhesiveness. The apparatus consisted of a double beam physical balance in which the right side has a pan, and the left side of the balance has a string that was hanged and at the bottom of the string was a suctioned glass slide. This was the place where the tablets were placed using an adhesive. The porcine buccal mucosa was placed on top of an inverted 50 mL beaker which was placed inside

Table 5: Evaluation parameters of different mucoadhesive tablets

Formulation	*%Drug content*	*%Water absorption*	*Mucoadhesive strength*
F1	92.99 ± 6.66	45.25 ± 2.20	15.26 ± 0.12
F2	95.29 ± 2.54	59.29 ± 3.26	16.29 ± 2.25
F3	97.98 ± 6.59	52.33 ± 2.25	17.21 ± 0.51
F4	90.85 ± 4.52	49.28 ± 2.25	15.54 ± 1.25
F5	92.95 ± 3.26	48.35 ± 0.53	19.51 ± 2.25

Table 6: Drug release of different formulations

Time in Hours	% Drug Release				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	40	30	10.8	15.5	30.3
2	55	50	35.8	26.2	45.4
4	60	70	45.5	46.5	60.3
6	75	100	65.5	68.5	85.5
8	100	-	80.2	75.4	100
12	-	-	90.2	85.2	.

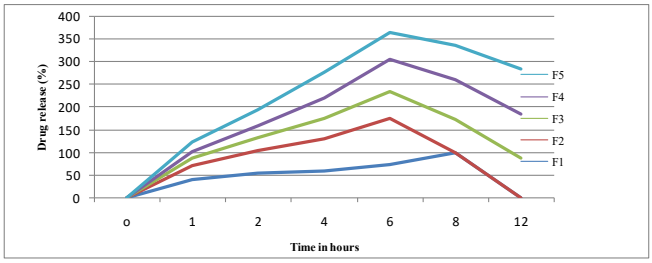

Fig. 1: Drug release for different formulation

Table 7: Swelling index of different formulation

Time in hours	Swelling index				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	10	5	15	20	5
2	25	10	20	30	15
3	35	12	35	35	20
4	50	18	60	48	35
5	55	25	75	60	45

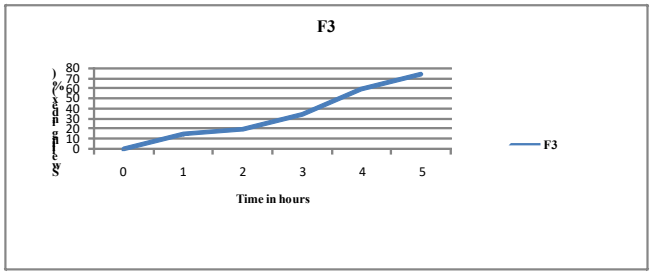

Fig. 2: swelling index (%) curve for F3 Formulation

a 500 mL beaker that was filled with phosphate buffer with pH 6.8 kept at 37 °C. The buffer amount was just enough so that it reaches the buccal mucosa surface. Exactly five gram of weight was placed on the right pan before putting the porcine buccal tablet in place. The weight was then removed to lower the glass slide with the attached buccal tablet. The tablet was to be in contact with the porcine buccal mucosa membrane and this was not disturbed for 5 minutes. After 5 minutes, weights were added on the right side of the pan to separate the tablet from the membrane. The accumulated weight on the right side was then noted and subtracted with 5 g. The value was taken as the measure for the bioadhesive strength of the tablet. The bioadhesive force was calculated using the formula:

$$N = W \times g \ 1000$$

Where N is bioadhesive force, W is the weight required for detachment of the tablet from the porcine buccal mucosa in grams, and g is the acceleration due to gravity at 9.81 m/sec2 (Fatima et al., 2015; Lodhi et al., 2013; Prasad et al., 2010). Figure 1 shows the modified physical balance.

Post-Compression Parameters of Mucoadhesive Tablets

Drug release In-vitro drug release studies were tested using

USP dissolution test apparatus II, the paddle type with dissolution medium of phosphate buffer with a pH of 6.8. It was performed at 37 °C + 0.5 °C with a speed of 50 rpm. The sample at 5 mL was withdrawn at time interval of 15, 30, 45, 60, 90, 120, 150, 180 minutes and was replaced with 5 mL of fresh phosphate buffer.

Conclusion

Mucoadhesive dosage forms have a high potential of being use full means of delivering drugs to the body for topical or local administration. The phenomenon of mucoadhesion can be used as a model for the controlled drug delivery approaches for a number of drug candidates. The various advantage of the oral mucoadhesive drug delivery systems like prolongation of the residence time of the drug which in turn increases the absorption of the drug are important factors in the oral bioavailability of many drug.

Mucoadhesive tablets in general have the potential to be used for controlled release drug delivery; mucoadhesive tablets has additional advantages, e.g. efficient absorption and enhanced bioavailability of the drugs, more intimate contact with the mucus layer. Mucoadhesive tablets are widely used because they release the drug for prolonged period, reduce the frequency of drug administration and improve patient compliance.

Ranitidine and amoxicillin is one of the best combinations of solid dosages form used for treating *H. pylori* infection. *H. pylori* is very sensitive to amoxicillin both *in-vivo* and *in-vitro*. Amoxicillin works by inhibition of bacterial cell wall synthesis, leading to cell death. Amoxicillin has topical intraluminal activity at the level of the gastric mucosa, as well as systemic activity. The advantage of amoxicillin is that *H. pylori* does not develop resistance to it, and it can be used again in another antibiotic regimen. Ranitidine is a histamine 2-receptor-antagonist (H2RA). Ranitidine works by reducing the amount of acid your stomach produces. The agent has been developed for the treatment of patients with duodenal ulcer with or without infection with *H. pylori*.

Combined ranitidine HCL with amoxicillin trihydrate has resulted in an eradication rate of 72 to 80% compared to triple antibiotic therapy. The food and drug Administration recently approved this combination. As mentioned earlier, the combination of ranitidine HCL with amoxicillin trihydrate has been shown to be effective in eradicating *H. pylori* infection.

Therefore, we have planned in the development of gastro retentive mucoadhesive tablet containing amoxicillin trihydrate and ranitidine HCL which may result in improved patient compliance, easy to administration, increased residence time resulting in enhanced absorption and increased therapeutic efficacy of the drug with reducing frequency of drug administration.

References

1. Alahdab YO, Kalayci C. *Helicobacter pylori*: Management in 2013, World Journal of Gastroenterology. 2013; 20(18): 5302-7.
2. Ahuja A, Khar RK, All J. Mucoadhesive drug delivery systems, Drug Development and industrial Pharmacy.1997: 489-515.
3. Bramankar DM, Jaiswal SB. Biopharmaceutics and Pharmacokinetics– A Treatise.1995:337-371.
4. Buzas GM. Helicobacter Polymer OxvHetil.2010:1956-151.
5. Bardonnet DL, Faivre V, Pugh WL, Affaretti JC, Falson F. Gastroretentive Dosage Forms : Overview and special case of *Helicobacter pylori*. Journal of Controlled Release.2006; 11(1-2): 1-18.
6. Chickering D, Jacob J, Mathiowitz E. 52 Poly (fumaric-cosebacic) Microspheres as oral drug delivery system. Biotechnology and Bioengineering.1996:96-101.
7. Das CJ, Paul N. (2006), Epidemiology and Pathophysiology of *Helicobacter pylori* Infection in children. Indian Journal of Paediatrics. 2006; 74(3):287-290.
8. De Vries AC, Kuipers EJ.*Helicobacter pylori* infection and non malignant disease. Helicobacter. 2010;15(9):1523-5378
9. Deshpande AA, Rhodes CT, Shah NH, Malick AW. (1996) Controlled Release Drug Delivery System for Prolonged Gastric Residence: an overview. Drug Development and Industrial Pharmacy.1996; 22:31-39.
10. Goh KL, Chan WK, Shiota S, Yamaoka Y. Epidemiology of *Helicobacter pylori* infection and public health implications. Helicobacter. 2011; 16 Suppl 1:1–9.
11. Gisbert JP. [*Helicobacter pylori*-related diseases] Gastroenterol Hepatol. 2012; 35 Suppl 1:12–25.
12. Umamaheshwari RB, Jain S, Jain NK. A new approach in gastroretentive drug delivery system using cholestyramine. Drug Deliv. 2003; 10:151–160.
13. 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.
14. Nagahara N, Akiyama Y, Nakao M, Tada M, Kitano M, Ogawa Y. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. Antimicrob Agents Chemother. 1998; 42:2492–2494.
15. Pawar VK, Kansal S, Garg G, Awasthi R, Singodia D, Kulkarni GT. Gastroretentive dosage forms: a review with special emphasis on floating drug delivery systems. Drug Deliv. 2011; 18:97–110.
16. Hwang SJ, Park H, Park K. Gastric retentive drug-delivery systems. Crit Rev Ther Drug Carrier Syst. 1998; 15:243–284.
17. Dunn BE, Cohen H, Blaser MJ. *Helicobacter pylori*. Clin Microbiol Rev. 1997; 10:720–741.
18. Marshall B. *Helicobacter pylori*: 20 years on. Clin Med. 2002; 2:147–152.
19. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med. 1991; 325:1127–1131.
20. Yamada T, Ahnen D, Alpers DH, Greenberg HB, Gray L, Joscelyn KB, Kauffman G, Podolsky DK, Ray WA, Schaberg

D, et al. Helicobacter-Pylori in Peptic-Ulcer Disease. Jama-J Am Med Assoc. 1994; 272:65–69.
21. Singh K, Ghoshal UC. Causal role of *Helicobacter pylori* infection in gastric cancer: an Asian enigma. World J Gastroenterol. 2006; 12:1346–1351.
22. Goodwin CS, Armstrong JA. Microbiological aspects of *Helicobacter pylori* (Campylobacter pylori) Eur J Clin Microbiol Infect Dis. 1990; 9:1–13.
23. Geis G, Suerbaum S, Forsthoff B, Leying H, Opferkuch W. Ultrastructure and biochemical studies of the flagellar sheath of *Helicobacter pylori*. J Med Microbiol. 1993; 38:371–377.
24. Semino-Mora C, Doi SQ, Marty A, Simko V, Carlstedt I, Dubois A. Intracellular and interstitial expression of *Helicobacter pylori* virulence genes in gastric precancerous intestinal metaplasia and adenocarcinoma. J Infect Dis. 2003; 187:1165–1177.
25. Krajewska B. Ureases I. Functional, catalytic and kinetic properties: A review. J Mol Catal B-Enzym. 2009; 59:9–21.
26. 17. Atherton JC. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. Annu Rev Pathol. 2006; 1:63–96.
27. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev. 2006; 19:449–490.
28. Blaser MJ, Atherton JC. *Helicobacter pylori* persistence: biology and disease. J Clin Invest. 2004; 113:321–333. Cover TL, Blaser MJ. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J Biol Chem. 1992; 267:10570–10575.
29. Guillemin K, Salama NR, Tompkins LS, Falkow S. Cag pathogenicity island-specific responses of gastric epithelial cells to *Helicobacter pylori* infection. Proc Natl Acad Sci USA. 2002; 99:15136–15141.
30. Genta RM, Graham DY. *Helicobacter pylori*: the new bug on the (paraffin) block. Virchows Arch. 1994; 425:339–347.
31. Cid TP, Fernández MC, Benito Martínez S, Jones NL. Pathogenesis of *Helicobacter pylori* infection. Helicobacter. 2013; 18 Suppl 1:12–17. Oertli M, Noben M, Engler DB, Semper RP, Reuter S, Maxeiner J, Gerhard M, Taube C, Müller A. *Helicobacter pylori* γ-glutamyl transpeptidase and vacuolating cytotoxin promote gastric persistence and immune tolerance. Proc Natl Acad Sci USA. 2013; 110:3047–3052.
32. Goodgame RW, Malaty HM, el-Zimaity HM, Graham DY. Decrease in gastric permeability to sucrose following cure of *Helicobacter pylori* infection. Helicobacter. 1997; 2:44–47.
33. Borch K, Sjöstedt C, Hannestad U, Söderholm JD, Franzén L, Mårdh S. Asymptomatic *Helicobacter pylori* gastritis is associated with increased sucrose permeability. Dig Dis Sci. 1998; 43:749–753.
34. Noach LA, Rolf TM, Tytgat GN. Electron microscopic study of association between *Helicobacter pylori* and gastric and duodenal mucosa. J Clin Pathol. 1994; 47:699–704.
35. McColl KE. *Helicobacter pylori* and acid secretion: where are we now? Eur J Gastroenterol Hepatol. 1997; 9:333–335.
36. Go MF, Kapur V, Graham DY, Musser JM. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J Bacteriol. 1996; 178:3934–3938.
37. O'Connor A, Gisbert JP, McNamara D, O'Morain C. Treatment of *Helicobacter pylori* Infection 2011. Helicobacter. 2011; 16 (Suppl 1):6.
38. Cavallaro LG, Egan B, O'Morain C, Di Mario F. Treatment of *Helicobacter pylori* infection. Helicobacter. 2006; 11 Suppl 1:36–39.
39. Gumurdulu Y, Serin E, Ozer B, Kayaselcuk F, Ozsahin K, Cosar AM, Gursoy M, Gur G, Yilmaz U, Boyacioglu S. Low eradication rate of *Helicobacter pylori* with triple 7-14 days and quadriple therapy in Turkey. World J Gastroenterol. 2004; 10:668–671.
40. Ogata SK, Godoy AP, da Silva Patricio FR, Kawakami E. High *Helicobacter pylori* resistance to metronidazole and clarithromycin in Brazilian children and adolescents. J Pediatr Gastroenterol Nutr. 2013; 56:645–648.
41. Gomollón F, Santolaria S, Sicilia B, Ferrero M, Revillo MJ, Ducóns J, Villar M, Celaya MC, Montoro M. [*Helicobacter pylori* resistance to metronidazole and clarythromicin: descriptive analysis 1997-2000] Med Clin (Barc) 2004; 123:481–485.
42. Mégraud F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. Gut. 2004;53:1374–1384.
43. Seddik H, Ahid S, El Adioui T, El Hamdi FZ, Hassar M, Abouqal R, Cherrah Y, Benkirane A. Sequential therapy versus standard triple-drug therapy for *Helicobacter pylori* eradication: a prospective randomized study. Eur J Clin Pharmacol. 2013; 69:1709–1715.
44. Lim JH, Lee DH, Choi C, Lee ST, Kim N, Jeong SH, Kim JW, Hwang JH, Park YS, Lee SH, et al. Clinical outcomes of two-week sequential and concomitant therapies for *Helicobacter pylori* eradication: a randomized pilot study. Helicobacter. 2013; 18:180–186.
45. Jafri NS, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive to treatment. Ann Intern Med. 2008; 148:923–931.
46. Tsay FW, Tseng HH, Hsu PI, Wang KM, Lee CC, Chang SN, Wang HM, Yu HC, Chen WC, Peng NJ, et al. Sequential therapy achieves a higher eradication rate than standard triple therapy in Taiwan. J Gastroenterol Hepatol. 2012; 27:498–503.
47. Molina-Infante J, Romano M, Fernandez-Bermejo M, Federico A, Gravina AG, Pozzati L, Garcia-Abadia E, Vinagre-Rodriguez G, Martinez-Alcala C, Hernandez-Alonso M, et al. Optimized nonbismuth quadruple therapies cure most patients with *Helicobacter pylori* infection in populations with high rates of antibiotic

resistance. Gastroenterology. 2013; 145:121–128.e1.
48. Molina-Infante J, Pazos-Pacheco C, Vinagre-Rodriguez G, Perez-Gallardo B, Dueñas-Sadornil C, Hernandez-Alonso M, Gonzalez-Garcia G, Mateos-Rodriguez JM, Fernandez-Bermejo M, Gisbert JP. Nonbismuth quadruple (concomitant) therapy: empirical and tailored efficacy versus standard triple therapy for clarithromycin-susceptible *Helicobacter pylori* and versus sequential therapy for clarithromycin-resistant strains. Helicobacter. 2012;17:269–276.
49. Hsu PI, Wu DC, Wu JY, Graham DY. Modified sequential *Helicobacter pylori* therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. Helicobacter. 2011; 16:139–145.
50. Kuo CH, Kuo FC, Hu HM, Liu CJ, Wang SS, Chen YH, Hsieh MC, Hou MF, Wu DC. The Optimal First-Line Therapy of *Helicobacter pylori* Infection in Year 2012. Gastroenterol Res Pract. 2012;2012:168361.
51. Sardarian H, Fakheri H, Hosseini V, Taghvaei T, Maleki I, Mokhtare M. Comparison of hybrid and sequential therapies for *Helicobacter pylori* eradication in Iran: a prospective randomized trial. Helicobacter. 2013;18:129–134.