European Journal of Pharmaceutical Sciences 84 (2016) 139–145

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Formulation and advantages of furazolidone in liposomal drug delivery systems

Muhammad Irfan Alam, Timothy Paget, Amal Ali Elkordy *

Sunderland Pharmacy School, Department of Pharmacy, Health and Well-being, University of Sunderland, Sunderland, UK

ARTICLE INFO

Article history:
Received 18 October 2015
Received in revised form 13 January 2016
Accepted 13 January 2016
Available online 18 January 2016

Keywords:
Mucoadhesion
Furazolidone
Liposomes
Cholesterol
Encapsulation efficiency
Drug release

ABSTRACT

Furazolidone has proven to have antiprotozoal and antibacterial activity. A number of literature supported its use against *Helicobacter pylori*. This potential application opens new prospects of its use in clinical settings in triple therapy. In order to avoid side effects associated with this drug, liposomal mucoadhesive drug delivery that can work locally in stomach is considered as an appropriate approach. This study is a focus on formulations and *in vitro* characterization of liposomes containing furazolidone. Therefore, the effects of variable amounts of drug and cholesterol on encapsulation efficacy and *in vitro* drug release were evaluated for different liposomal formulations. Mucoadhesive behavior of chitosan coated liposomal at two different pHs was also evaluated and increase in pH from 1.3 to 4.5 increased mucoadhesion from 42% to 60% respectively. Increasing the amount of drug from 4 mg to 5 mg increased encapsulation activity however, increasing the drug any further decreased encapsulation activity. In contrast, by increasing the amount of cholesterol decrease in encapsulation activity was observed. The optimized formulation with 5 mg of drug and 53 mg of cholesterol in formulation gave 57% drug release at pH 1.3 but release was increased up to 71% by increasing pH to 4.5 for same amount of drug. However, by using 10.6 mg of cholesterol and 5 mg of drug the overall release was increased at both pH conditions, at pH 1.3 release was 69% as compared to 77% at pH 4.5. This trend of drug release profile and mucoadhesion that favors pH 4.5 is documented as useful in targeting *H. pylori* as normal pH of stomach is expected to be higher by the influence of this microbe. Hence, the results of this research can be taken further into a future *in vivo* study.

1. Introduction

Helicobacter pylori is a gram-negative bacteria that inhabits in microaerophilic environment. It is considered as a major cause of peptic ulcer as well as chronic gastritis and gastric carcinoma (Blaser and Atherton, 2004; Cover and Blaser, 2009; Warren and Marshall, 1983). The current therapy for eradication of *H. pylori* is a triple therapeutic regimen that involves two antibiotics (Hidekazu et al., 2015) and one proton pump inhibitor, but this presently established therapeutic regimen is not absolutely effective and results in incomplete eradication in most of the cases. One of the major important issue in treatment of *H. pylori is* antibiotic resistance (Arora et al., 2012).

In order to deal with resistance problem different combinations of antibiotics in triple therapy are being used and furazolidone that is monoamine oxidase inhibitor presents very low resistance for therapeutic regimen. There are studies demonstrating its efficacy (Aliakbar et al., 2015) and safety in several developing countries against *H. pylori* (Venkateswaramurthy et al., 2010). But unfortunately the use of furazolidone is limited due to potential side effects associated with it because of its high dose of administration and the problem must be addressed in order to unveil the high potential antimicrobial activity of furazolidone. Accordingly, the aim of the current study to minimize the side effects associated with drug is endeavored by using two communal strategies: using the minimum required amount of drug in liposomal drug delivery carriers as compared to high conventional dose and secondly use of mucoadhesive approach for liposomes in order to release drug locally in stomach.

Liposomes are small vesicles that can accommodate both hydrophilic and hydrophobic drugs and have been under consideration for drug delivery from the last few decades. They can be used potentially not only for ocular drug delivery systems (Li et al., 2009; Habib et al., 2010) but also for oral drug delivery systems because of their fragile nature and short half-life as well as they protect encapsulated drug from degradation in the stomach (Jesorka and Orwar, 2008; Parmentier et al., 2010). A number of attempts have been made to increase the gastric retention of the drug and mucoadhesion is one of the promising approaches for that. Delayed gastric retention can enhance bioavailability for the local delivery of drugs to the stomach (Ankit and Akhlesh, 2013). Therefore the current study is focused on mucadhesive liposomal preparation by using chitosan as mucoadhesive polymer. Chitosan is a polysaccharide and has got a number of advantages like low toxicity, biocompatibility and mucoadhesive property that enable it to be

* Corresponding author at: University of Sunderland, Department of Pharmacy, Health and Well-being, Sunderland SR1 3SD, UK.
E-mail address: amal.elkordy@sunderland.ac.uk (A.A. Elkordy).

http://dx.doi.org/10.1016/j.ejps.2016.01.017
0928-0987/

considered as a potential candidate for mucoadhesive formulations (Karn et al., 2011).

To the best of our knowledge, no efforts have been made to encapsulate furazolidone in mucoadhesive liposomal drug delivery systems.

2. Materials and methods

2.1. Materials

Furazolidone, cholesterol and chitosan were purchased from sigma Aldrich. Phosphatidylcholine (Egg PC 80 E S) was given as a gift sample from Lipoid Switzerland. Coumarin-6 was purchased from fisher scientific UK. Mucin Type III and pepsin (partially purified from porcine stomach) were purchased from sigma Aldrich. All other chemicals, reagents and solvents used were of either analytical or pharmaceutical reagent grade.

2.2. Liposome preparation

Liposomes were prepared by thin film hydration method using different ratios of cholesterol (Bhatia et al., 2004). Accurately weighed phosphatidylcholine, cholesterol and cremophore ELP as co-surfactant were dissolved in 8 ml of chloroform. However, furazolidone was dissolved in 3 ml of acetonitrile. After dissolving, choloroform–acetonitrile mixture containing drug and other constituents was transferred in 100 round bottom flask and organic solvents were evaporated by rotary evaporator (Buchi RE 121 Switzerland) at 60 °C. After evaporation thin film on inner surface of flask was flushed with nitrogen gas for 10 min to remove the traces of organic solvent followed by rehydration of film by 5 ml trizma buffer pH 7.4 at 53 °C for half an hour. Different variables considered in this study have been presented in (Table 1).

2.3. Mucoadhesive liposomes

Liposomes were prepared by conventional film hydration method (Section 2.2) with coumarin-6 as fluorescent dye instead of furazolidone and then coated by chitosan for mucoadhesion (Table 2). Equal volumes of liposomal suspension and 0.6% *w*/*v* solution of chitosan in 0.1% *v*/v glacial acetic acid were mixed at a rate of 1 ml per minute by continuous stirring at 25 °C. Resulting suspension was kept in refrigerator overnight.

2.4. Mucoadhesion analysis

2.4.1. Fluorimetry

Mucoadhesion analysis was performed at two different pHs i.e. 1.3 and 4.5. Freshly excised stomach of sheep was cut into 2 × 2 cm slices. The volume of 100 μl of liposomal suspension (LC_1, Table 2) was spread onto each tissue specimens. Each tissue specimen was placed in 5 ml vial separately containing simulated gastric fluid (SGF) that contained 0.1% pepsin (sigma P-700), 0.1% Mucin (sigma type III), 20.5 mmol NaCl and 2.7 mmol KCl, and 0.1 M HCl adjusted at the required pH (O'Gara et al., 2008). Vials were put on a shaker incubator (50 rpm) at 37 °C. Tissue specimens were taken out at predetermined time intervals (0, 1, 1.5, 3, 4.5, 6 h) and rinsed with 10 ml of phosphate buffer saline (PBS) to remove unadsorbed liposomes. Mucus was removed carefully and put in 5 ml of 5 M NaOH solution for 12 h to dissolve mucus or any traces of tissue completely. Isopropyl alcohol (IPA) and acetic acid was added to samples to disrupt the lipid membrane and dissolve chitosan followed by centrifugation at 6000 rpm for 10 min to extract coumarin-6 from liposomes. Supernatant was removed and intensity was measured by fluorimeter and percentage of dye recovered from stomach tissue was determined by using calibration curve of coumarine-6. Non mucoadhesive liposomal suspension (NLC_1, Table 2) was used as a control for comparison and results of both formulations were compared.

Table 2
Composition of fluorescence labeled liposomes.

Formulation	Composition	Mucoadhesive liposomes
	Coumarin6:lipid:cholesterol (weight)	Chitosan
LC_1	2.5 μg:26.5 mg:2.5 mg	0.6% (*W*/*V*)
NLC_1	2.5 μg:26.5 mg:2.5 mg	0

LC_1: Liposomes containing coumarin-6 with chitosan; NLC_1: liposomes containing coumarin-6 without chitosan.

2.4.2. Fluorescence microscopy

Freshly excised sheep stomach was cut into 0.5 × 0.5 cm slices. Each slice was coated with 0.5 ml of mucoadhesive liposomal suspension (LC_1, Table 2) that contained coumarin-6 as fluorescence dye. Slices were incubated in 5 ml SGF at required pH i.e. 1.3 and 4.5. Vials were put on a shaker incubator (50 rpm) at 37 °C. Tissue specimens were taken out at different time intervals over 6 h. Then, tissue specimens were immediately frozen by snap freezing by using liquid nitrogen and OCT into a block. Each specimen was cut into 10 μm slices by using cryostat (LEICA, Germany) and observed under fluorescence microscope. The specimens were observed under the microscope for the number of liposomal particles attached to the stomach slice after removing from SGF and quantified by comparing specimens at each specified time intervals.

2.5. Encapsulation efficiency

Encapsulation efficiency of liposomes was determined where liposomal suspension containing liposome bounded as well as free drug was centrifuged at 4 °C for 10 min at 15,000 rpm and three layers were generated. Supernatant in the first layer was discarded and second layer of liposomes were removed by using micropipette without disturbing third yellow colored layer of the un-entrapped drug. Liposomes were transferred into new eppendorf and washed with distilled water followed by re-centrifugation. The cycle of washing and centrifugation was repeated three times and liposomal pellets without any free drug were

Table 1
Effect of cholesterol content and drug on encapsulation efficiency (EE) and drug loading.

Formulation	Drug (mg)	Lipid: cholesterol (weight/mg)	% EE	Drug entrapped (mg)
L1	4	106:53	43.3	1.73
L2	5	106:53	44.7	2.23
L3	8	106:53	29.9	2.39
L4	4	106:10.6	45.7	1.82
L5	5	106:10.6	47.2	2.36
L6	8	106:10.6	35.4	2.83

Particles size, nm, measurement of liposomes with highest encapsulation efficiency, L5.

	Nanopore	Mean	Mode	Max	Min	Particle count
L5	200	535	374	2082	266	435
	400	692	482	2236	328	1070