International Journal of Pharmaceutics 493 (2015) 295–304

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Gastroretentive montmorillonite-tetracycline nanoclay for the treatment of *Helicobacter pylori* infection

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ARTICLE INFO

Article history:
Received 17 March 2015
Accepted 25 June 2015
Available online 31 July 2015

Chemical compounds studied in this article:
Magnesium aluminum silicate (PubChem CID: 3084116)
Tetracycline hydrochloride (PubChem CID: 54704426)
Cesium chloride (PubChem CID: 24293)
Chitosan (PubChem CID: 71853)

Keywords:
Helicobacter pylori
Montmorillonite
Tetracycline
Nanoclay
Mucoadhesion

ABSTRACT

The paper aims to explore the potential benefits provided by an organically modified montmorillonite (nanoclay) in the problematic management of the *Helicobacter pylori* gastric infection that is one of the most prevalent infectious diseases worldwide. Two nanoclay samples were produced by the intercalation of tetracycline (TC) into the interlayer of montmorillonite (MM) under two different pH reaction conditions (pH 3.0 and 8.7). MM/TC nanoclays were characterized by EDX, XRD, FTIR, DSC, drug adsorption extent, *in vitro* mucoadhesiveness and desorption in simulated gastric media. The reaction between MM and TC led to a complete MM cation (Na^+ and Ca^{2+}) exchange process, an increase of MM characteristic interlayer spacing as well as an involvement of NHR_3^+ group of TC, regardless of the reaction pH value. However, MM/TC nanoclay obtained under alkaline conditions provided a lower TC adsorption as well as a drug fraction weakly linked to MM in comparison with the nanoclay obtained in acidic conditions. Both the nanoclays exhibited good mucoadhesion properties to porcine mucin and TC desorption occurring mainly *via* a cation exchange process by H^+ ions. Based on the results obtained, TC intercalation into MM nanoplatelets could represent a potential advantageous approach allowing the antibiotic to distribute homogeneously on the gastric mucosa, diffuse through the gastric mucus layer and achieve the microorganism localization.

1. Introduction

Helicobacter pylori (Hp) gastric infection is one of the most prevalent infectious diseases worldwide with an estimation of more than 50% of the world population. Hp infection is associated with several gastroduodenal pathologies such as chronic gastritis, gastric and duodenal ulcers. Furthermore, oxidative and nitrosative stresses in combination with inflammation observed in Hp-infected patients play an important role in gastric carcinogenesis, being Hp estimated to be responsible for approximately two-thirds of gastric lymphomas (Gisbert and Calvet, 2013). For these reasons, the Food and Drug Administration (FDA) has recently added Hp to the list of qualifying pathogens that have the potential to pose a serious threat to public health (FDA, 2014).

In accordance with FDA and European Maastricht guidelines (Malfertheiner et al., 2012), the recommended therapy involves different regimens based on the oral administration of two or more antibiotics combined with proton pump inhibitors or antacids twice a day for up to 14 days, and in fed conditions to increase the gastric residence time of the drug. However, this latter condition is relevantly affected by inter-individual differences in gastric emptying as well as by the amount and variety of the ingested food. Even with a right selection of drugs, bacterial eradication may fail in up 20–40% of patients owing to antibiotic resistance, insufficient antibiotic concentration reaching the site of infection (under the gastric mucus gel layer), uneven drug distribution in the gastric lumen, short contact time between drug formulation and gastric mucosa, and poor patient compliance due to the complex therapeutic regimen which also produces side effects (Gasparetto et al., 2012; Siddalingam and Chidambaram, 2014; WGO Global Guidelines, 2010). Among these difficulties connected with Hp cure, failure of therapy is mostly associated to antibiotic resistance and patient non-compliance (Graham and Shiotani, 2008). Indiscriminate use of antibiotics combined with non-adherence to therapy have led to a growing increase in the resistance of bacteria to antibiotics, particularly macrolides, fluoroquinolones and metronidazole (Kim et al., 2001; Malfertheiner et al., 2012;

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http://dx.doi.org/10.1016/j.ijpharm.2015.06.049
0378-5173/

Oleastro et al., 2011; Papastergiou et al., 2014; Siavoshi et al., 2010). Another reason for the failure of the conventional therapy lies in Hp nature. Hp colonization occurs because the microorganism survives in the acidic gastric environment owing to its urease activity producing ammonia and bicarbonate from urea. After that, Hp adheres to the gastric epithelium under the mucus gel layer where H^+ ions are neutralized by HCO_3^- secreted by the epithelial cells (Allen and Garner, 1980; Henriksnäs et al., 2006). Therefore, access for antimicrobial drugs to the infected site is limited by both the acidic gastric lumen affecting drug stability and mucus gel layer which hinders the attainment of sufficient antibiotic concentrations for bactericidal activity (Lopes et al., 2014; Umamaheshwari et al., 2004). Therefore, a new treatment approach that is also simple to encourage patient compliance aiming to achieve higher eradication rates is urgently needed (Gatta et al., 2013; Siddalingam and Chidambaram, 2014; Vakil and Vaira, 2013). On these assumptions, a stomach-site specific drug delivery system would increase the localized concentration and the residence time of the drugs at the site of action making less variable the gastric emptying time, reducing dosage frequency, and increasing patient compliance. For this reason, scientific research has proposed floating or mucoadhesive systems that prolong intragastric residence time, gradually releasing the drug in the gastric region for a longer time than normal gastric emptying, requiring a single dose per day and being scarcely influenced by food ingested. In addition, the entire dose could be released in the stomach resulting in an increased efficacy of the formulation. Most of these studies involved multiparticulate delivery systems able to be distributed over a wider surface of the gastric mucosa in comparison with monolithic devices (Adebisi and Conway, 2014; Bardonnet et al., 2006; Ishak et al., 2007; Liu et al., 2005; Patel and Patel, 2007; Sahasathian et al., 2010). Concerning the mucoadhesion approach, animal model studies demonstrated that mucoadhesive microparticulate systems can reside in the stomach even after 10 h from the administration and that the transport of an antibiotic from the gastric lumen through the mucus layer is more effective than drug absorbed through the basolateral membrane from blood circulation as well as drug administered by means a conventional formulation (Prasanthi et al., 2011; Siddalingam and Chidambaram, 2014; Umamaheshwari et al., 2004).

On the basis of these premises, the purpose of this study was to exploit the use of a clay material from the smectite family in order to produce a mucoadhesive organically modified clay by means of the interaction between the clay and an anti-Hp antibiotic. Smectites are 2:1 (tetrahedral:octahedral sheet) layered silicates, characterized by octahedral and tetrahedral substitutions and high Cation Exchange Capacity (CEC), so they may undergo ion exchange with basic drugs in solution within their interlayer space. Smectite ability to exchange cations with several organic compounds has been used for five decades, and more recently, they have been proposed as materials for modulating drug delivery or improving dissolution of poorly water-soluble drugs (Aguzzi et al., 2005; Joshi et al., 2009). In addition to these properties, smectites such as clays in general possess strong bioadhesive properties, and gastroprotective antacid activity due to their interaction with the mucus glycoproteins neutralizing the gastric acidity (Droy-Lefaix and Tateo, 2006). Among smectites, montmorillonite (MM), the main component of bentonite, could be considered as the essential clay material, known to have platelet structure with average dimension of 1 nm thick and 70–150 nm wide from which nanoclay definition originates (Patel et al., 2006; Uddin, 2008). Montmorillonite is mainly used as auxiliary material in the pharmaceutical industry for oral or topical dosage forms, recorded in the United States Pharmacopoeia, European Pharmacopoeia, and British Pharmacopoeia.

Therefore, an organically modified MM nanoclay was developed by intercalating tetracycline (TC) into MM nanoplatelets. TC was used in the first effective therapies against Hp and is currently used as part of second-line therapy (triple or quadruple regimen) eradicating Hp. Hp isolated from 138 patients resulted sensitive to TC with a MIC $\leq$ 0.38 mg/L and Hp resistance to TC is considered as rare in most countries owing to a stepwise process involving a triple-base-pair substitution within Hp rRNA genes (Gerrits et al., 2003; Samra et al., 2002). Adsorption of tetracyclines onto soils and clay minerals were conducted as early as 1950s even if to a limited extent. To date these studies focused on removal TC as an organic contaminant of water or soils to improve environmental quality (Aristilde et al., 2013; Avisar et al., 2010; Chang et al., 2009; Wang et al., 2010), and on biopharmaceuticals effects due to the co-administration of TC and clays acting as suspending agents (Browne et al., 1980; Porubcan et al., 1978), or topical excipients (Parolo et al., 2010). Conversely, clays have not been hitherto object of study for the development of a gastroretentive TC delivery system.

In the present work, MM-TC nanoclays were obtained under two different pH reaction conditions and characterized for the interaction occurred between drug and nanoclay, drug adsorption and *in vitro* desorption extents. In particular, a novel application of Energy Dispersive X-ray (EDX) analysis is proposed to determine clay Cation Exchange Capacity (CEC) giving more information about the elements involved in the intercalation process compared with the current methods. Moreover, the nanoclay samples were evaluated *in vitro* for mucoadhesiveness in a perspective of a gastroretentive formulation able to improve the performance of Hp infection therapy.

2. Materials and methods

2.1. Materials

For the organically modified nanoclay preparation, montmorillonite (MM) [$(NaCa)_{0.33}(AlMg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$), Veegum R, Magnesium Aluminum Silicate NF, pharmaceutical grade, >90% montmorillonite] from Vanderbilt Minerals, LLC (Norwalk, CT, USA) and tetracycline hydrochloride (TC, MW 480.90) from Fluka Chemie (Buchs, Switzerland) were purchased. For Cation Exchange Capacity (CEC) determination, cesium chloride, CsCl, was purchased from Sigma–Aldrich (Milan, Italy). For the mucoadhesion assay, chitosan (low molecular weight, >75% deacetylated, viscosity 20–300 cps in 1% acetic acid at 25 °C) from Fluka Chemie and mucin from porcine stomach, type II from Sigma–Aldrich were purchased. All the other chemicals were of analytical grade.

2.2. Montmorillonite CEC determination

Montmorillonite Cation Exchange Capacity (CEC) was measured by placing 100 mg clay in a 0.1 M solution of CsCl (125 ml) under magnetic stirring for 24 h. After centrifugation (3000 rpm for 15 min), the powder was rinsed with water, vacuum dried, compressed in a hydraulic press (PerkinElmer) at 200 kg/cm^2 for 1 min using 12.5 mm diameter punches, mounted on carbon stubs without conductive coating, and assayed by Energy Dispersive X-ray (EDX, Oxford INCA-350, FEI Company-Oxford Instruments, Oregon, USA) analysis coupled with an Environmental Scanning Electron Microscopy (ESEM, Quanta 200 Fei Company-Oxford Instruments) by the selected area method. EDX analysis is a technique usually used to identify the elemental composition of a sample. In this case, it allowed determining clay CEC, exploiting the peculiar affinity of phyllosilicates for some elements, among which cesium. X-ray emission of Cs atoms was evaluated at the intensity characteristic of this element (Lα = 4.2865 keV) and at the following working conditions: acceleration voltage 12 kV, spot size of 3, detection limit <0.3%. Moreover, X-ray emissions from the atoms calcium, potassium, carbon, oxygen, sodium, magnesium,