Journal of Drug Delivery Science and Technology 30 (2015) 494–500

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Pectin beads loaded with chitosan–iron microspheres for specific colonic adsorption of ciprofloxacin

Franceline Reynaud [b], Nicolas Tsapis [a], Silvia S. Guterres [c], Adriana R. Pohlmann [c], Elias Fattal [a, *]

[a] Univ Paris-Sud, CNRS UMR 8612, Institut Galien Paris-Sud, Labex LERMIT, 5 rue Jean-Baptiste Clément, Châtenay-Malabry, France
[b] Univ Federal Rio de Janeiro, Faculdade de Farmácia, Ilha do Fundão, CCS, 21941-590 Rio de Janeiro, RJ, Brazil
[c] Univ Federal Rio Grande do Sul, Faculdade de Farmácia, Av. Ipiranga, 2752, 90610-000, Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 1 June 2015
Received in revised form
20 July 2015
Accepted 20 July 2015
Available online 21 July 2015

Keywords:
Pectin
Chitosan
Iron
Colon delivery
Iron
Adsorption
Antibiotics

ABSTRACT

Residual antibiotics reaching the colon can induce severe deleterious effects on the colonic microbiota including the induction of resistance to antibiotics. To avoid the selection of ciprofloxacin resistance, chitosan–iron microspheres were encapsulated into pectin beads with the aim to specifically adsorb residual colonic ciprofloxacin, reducing its time of contact with the endogenous bacteria. In our study, the formulation of beads, their stability as well as their capacity to specifically adsorb ciprofloxacin in simulated digestive media was carried out. Beads incubated in simulated gastric and intestinal media were stable for 1 and 5 h, respectively. When incubated in simulated colonic medium, beads were then degraded by pectinases present in the medium. Coating with Eudragit® RS was needed to prevent the early adsorption of the antibiotic in intestinal medium. Adsorption studies in simulated colonic medium show that the adsorption capacity of chitosan–iron is not modified after encapsulation within pectin beads making clinically feasible the sequestration of residual ciprofloxacin reaching the colon.

1. Introduction

Since the introduction of penicillin into human therapeutics and throughout the past decades, antibiotics have been used and overused generating a strong selective pressure, which has resulted in the survival and spread of resistant bacteria [1,2]. This situation applies to antibiotic administration both in animals and humans where they are used for therapy and prophylaxis of infectious diseases [3].

Most bacteria have multiple routes to resistance to any antibiotic and, once resistant, they can rapidly give rise to vast numbers of resistant progeny [4]. Emergence of resistance in bacteria can result from two different sequences of events, including either a direct one-step selection of resistant clones at the site of infection, or an indirect two-steps process in which commensal resistant bacteria are first selected in the natural ecosystems of humans. They can then transfer resistance mechanisms horizontally to pathogenic species [1,5]. This is the reason why there is a growing concern about the increased prevalence of antibiotic resistance which has become a major issue of public health [6].

Resistance is a significant cause of excess morbidity, mortality, and cost. Numerous reports have emphasized the need to pursue campaigns for lower and better use of antibiotics in order to maintain their effectiveness [7]. However, the past decades experience has demonstrated that new antibiotics may be effective for a restricted period only. Resistance will develop sooner or later for each new drug. In an attempt to maintain the use of current antibiotics, a general consensus has been achieved in many meetings on the need of a large reduction in the use of presently available antibiotics [8]. However, in clinical practice, such promises are of little or no use since they are easily forgotten and neglected. There has been no change since early studies on the efficiency of antibiotic therapy, when development of resistance was reported along with the need of restricted use [4].

It becomes therefore urgent to search for novel approaches when dealing with the antibiotic multi-resistance issue. Several studies from our group have shown the efficacy of the oral administration of β-lactamase enzymes to protect the intestinal microbiota against residual β-lactams [9–12]. Another study has

* Corresponding author.
E-mail address: elias.fattal@u-psud.fr (E. Fattal).

http://dx.doi.org/10.1016/j.jddst.2015.07.014
1773-2247/

demonstrated the potentiality of non-enzymatic intra-intestinal inactivation of residual ciprofloxacin by colon-targeted zinc-pectinate beads loaded with activated charcoal [13,14]. This approach has recently been applied with success in a phase I clinical trial [15]. This system is however not selective to the antibiotics and non-specific adsorption of other molecules can occur interfering with major physiological processes. In a previous report, we have shown that chitosan–iron (CH–Fe) microspheres are capable to adsorb ciprofloxacin, an antibiotic often responsible of emergence of antibiotic resistance [16]. Furthermore, we demonstrated that the adsorption mechanism of ciprofloxacin by CH–Fe(III) is controlled by chemisorption. The aim of the present study is therefore to design intestinal colon-targeted zinc-pectinate beads loaded with chitosan–iron microspheres to selectively inactivate residual ciprofloxacin present in the colonic medium. We believe this system could be applied to any other undesired drug, present in the colon, presenting a high affinity to chitosan–iron microspheres.

2. Material and methods

2.1. Materials

Amidated low methoxylated (LM) pectin (Unipectine™ OG 175C, degree of esterification from 22% to 28% and degree of amidation from 19% to 23%) (Mw 120 kDa) was a gift from Cargill France (France). Chitosan, zinc acetate dihydrate, ciprofloxacin and polyethylene glycol 300 (PEG) were obtained from Fluka (Switzerland). Pancreatin from porcine pancreas and Pectinex® SP-L (26000 PG/mL), a mixture of pectinases from *Aspergillus aculeatus* were purchased from Sigma–Aldrich (Saint-Louis, USA). Eudragit® RS was provided by Evonik (Germany) and Glutaraldehyde from VWR International S.A.S (Fontenay-sous-bois, France). Sodium chloride was obtained from Merck Eurolab (Strasbourg-France). Nimesulide, Phosphate buffer saline, hydroxyethylpiperazine-ethanesulfonic acid (HEPES), 1-(2-pyridylazo)-2-naphthol (PAN), ammonia buffer (pH 10) for complexometry and iron(III) nitrate were purchased from Sigma–Aldrich (Saint-Louis, USA).

Simulated intestinal media were prepared according to the US Pharmacopeia XXVI Edition as described previously [14]. They were composed accordingly as followed: *Simulated gastric medium* (SGM): NaCl 30 mM, concentrated HCl (~12 M) was added to adjust pH to 1.2. *Simulated intestinal medium* (SIM): HEPES buffer 30 mM containing 1% pancreatin. The pH was adjusted to 6.8 with NaOH 0.2 M. *Simulated colonic medium* (SCM): HEPES/NaCl 10 mM/145 mM, containing 5200 PG/mL of pectinolytic enzymes. The pH was adjusted to 6.0 with NaOH 1 M.

2.2. Chitosan–iron microspheres

Chitosan–iron (CH–Fe) microspheres were prepared and characterized according to a procedure previously described [16]. Briefly, chitosan was dissolved in an aqueous solution of $Fe(NO_3)_3$ 0.1 M and stirred for 12 h to allow the formation of a chitosan–iron complex. The solution was then spray-dried in a Büchi-B191 mini spray-dryer (Büchi, Switzerland) with a standard 0.7 mm nozzle. The spray-drying conditions regarding spray flow, inlet temperature and air flow rate were set at 5 mL min^{-1}, 150 °C, 600 L h^{-1}, respectively. Afterwards, CH–Fe microspheres were cross-linked, as described [16], with glutaraldehyde (in solution in acetone at 25%) at a molar ratio of 1/1 (chitosan:glutaraldehyde) under magnetic stirring for 4 h in acetone. Microspheres were then filtered on a sintered glass funnel (pore size 1–1.6 μm) and washed with an ethanol-water (2/1, v/v) solution to remove the excess of $Fe(NO_3)_3$ and glutaraldehyde. Finally, they were vacuum-dried in a desiccator for at least 24 h. Pure chitosan microspheres (CH) were prepared using the same conditions without introducing $Fe(NO_3)_3$.

2.3. Encapsulation of microspheres within pectin beads

Zinc pectinate beads were prepared by ionotropic gelation according to a modified procedure described previously [14]. On one hand, a pectin solution in distilled water was obtained [concentration from 3 to 6% (w/v)]. On the other hand, CH–Fe microspheres were suspended in water at a concentration ranging from 3 to 5% (w/v). Equal volumes of the solution and the suspension were mixed using a vortex (Whirlimixer, England). Several CH–Fe/pectin ratios were considered. Beads were then formulated by adding the mixture drop-wise into a 12% (w/v) zinc acetate solution, using a syringe pump and a nozzle of 0.8 mm inner diameter. By contact with zinc ions, pectin droplets instantaneously turned into gelled beads. In order to obtain complete gelification, beads were maintained in the zinc acetate solution for 30 min under stirring, and separated by filtration. To remove the excess of free zinc that does not participate to the pectin network, beads were washed into distilled water three times for 1 min. Finally, they were dried at 37 °C for 12 h.

To increase their stability in intestinal medium, beads were optionally coated with Eudragit® RS. 1 g (≈700 beads) were spray-coated with a mixture of Eudragit® RS and PEG 300 (5/1 w/v) dissolved into acetone/ethanol solutions (2/1, v/v). They were placed in a coating pan (inner diameter 10 cm) and the speed was adjusted to 20 rpm. The coating solution was air-sprayed manually using a hand-held spray gun. A stream of drying air at 37 °C was applied. Coating was performed until 20% weight gain was achieved.

2.4. Bead diameter

The mean diameter of 20 uncoated and Eudragit® RS-coated beads was determined by optical microscopy (Leitz Diaplan, France). The size of each individual bead was calculated using a calibrated glass slide. Results are expressed as the mean diameter (mm) ± standard deviation.

2.5. Scanning electron microscopy

The surface and cross-sectional morphologies of the dried beads (Eudragit® RS-coated and uncoated) were examined using scanning electron microscopy (LEO 9530, Gemini, France) at accelerating voltage of 3 kV. Cross-sections samples were prepared by fracturing beads manually. Prior to observation, samples were mounted on metal stubs, using a double-sided adhesive tape, and coated with a 4 nm platinum/palladium layer under vacuum (Cressington 208 HR, Eloise, France).

2.6. Swelling studies

The swelling behavior was determined gravimetrically on beads (1 bead mL^{-1}) that were placed in SIM (pH 6.8) at 37 °C and weighed 30, 60, 120, 180, 240 and 300 min after incubation. Weighting was achieved after drying the swollen beads carefully with filter paper to remove the excess of medium. Swelling (%) was expressed, in triplicate as follows:

$$Swelling\ (\%) = [(W_t - W_o)/W_o] \times 100$$

where W_o is dry bead weight and W_t is the weight of incubated bead at determined time after incubation in SIM (pH 6.8).