Colonic Delivery of β-Lactamases Does not Affect Amoxicillin Pharmacokinetics in Rats

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Received 13 February 2007; revised 3 May 2007; accepted 22 May 2007

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21115

ABSTRACT: Pectin beads containing β-lactamases were designed for the hydrolysis of colonic residual antibiotics responsible for the emergence of resistance. Beads were prepared by ionotropic gelation in $CaCl_2$ and stabilized by coating with polyethylenimine (PEI) to resist disintegration in the upper GI tract. Particle characterization showed that dried beads had a diameter around 1 mm independently of the presence of PEI. Seven to ten percent (w/w) of PEI was located on bead surface forming a coating layer as observed by scanning electron microscopy. PEI improved considerably bead stability in simulated intestinal medium while affecting slightly the encapsulation efficiency of active β-lactamases. Coated beads were able to preserve β-lactamases from premature leakage in the upper GIT whereas, in simulated colonic medium, pectinases induced matrix degradation and reduction of β-lactamase content especially in beads coated in a 0.8% PEI solution. Finally, the pharmacokinetics of amoxicillin in rat after oral administration was not modified by the co-administration of beads containing β-lactamases. In conclusion, PEI-coated beads are stable in the upper GIT but remain sensitive to the action of pectinolytic enzymes allowing release of β-lactamases in a colonic medium without modification of the absorption of a β-lactam antibiotic when co-administered with loaded beads. J Pharm Sci 97:1853–1863, 2008

Keywords: pectin beads; β-lactamases; colon delivery; coating; polyethylenimine

INTRODUCTION

Colon-specific drug delivery systems have raised a large interest for the oral administration of peptides, proteins or other labile drugs.[1] Indeed, the peptidase activity is lower in the colon than in the upper GIT and the residence time is very long.[2,3] Therefore, colon delivery might overcome the enzymatic barrier that hampers the oral administration of proteins and would allow to obtain locally high amounts of active molecules.

To achieve colon-specific delivery, various approaches have been developed. Among them, bacterially triggered delivery systems appear as the most selective. Through this mechanism, colon delivery can be achieved by exploiting the microbial enzyme activity predominantly present in that location.[4] A large number of polysaccharides are degraded by colonic enzymes and may form the basis for a suitable colon delivery system.[5] Among them, pectin, which is a naturally

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Journal of Pharmaceutical Sciences, Vol. 97, 1853–1863 (2008)

occurring polysaccharide of linear chains of α(1-4)-D-galacturonic acid, is non toxic, not digested by gastric or intestinal enzymes and almost totally degraded by pectinolytic enzymes produced by the colonic microflora. For these reasons, pectin is a highly interesting polymer for site-specific colon delivery.[6,7] Low methoxylated pectin, amidated or not, can form a gel in presence of divalent cations such as calcium. Through this mechanism, pectin has been used to prepare Ca-pectinate gel beads by ionotropic gelation with calcium ions[8] a mild process ideal for the encapsulation of proteins.[9,10] In a previous study, we have entrapped β-lactamases in Ca-pectinate beads and defined the optimal conditions for preserving their activity during the preparation process.[11] The oral administration of beads containing β-lactamases in a mice model induces an increase of these β-lactams inactivating enzymes in the faeces which was not observed after administration of free β-lactamases.[12] Since the presence of residual antibiotics in the colon leads to a disruption of the colonic microflora and consequently to the reduction of colonization resistance,[13] colonic delivery of β-lactamases should protect the local microflora against the spreading of resistant bacterial strains in the environment without affecting antibiotic pharmacokinetics.[14] However, despite a good release of β lactamases in the faeces, formulations needed to be optimized in order to achieve an optimal site specific delivery process and avoid a premature degradation of the co-administered antibiotic. Indeed, it is noteworthy that swelling properties of Ca-pectinate beads in aqueous media make them unable to efficiently avoid drug leakage during the transit through the upper GIT and imply the use of a coating agent to preserve their integrity until they reach the colon. For this purpose, we stabilized Ca-pectinate beads loaded with β-lactamases in the upper GIT by coating with a cationic polymer, polyethylenimine (PEI). We found that this good stability in the upper GIT did not modify the pharmacokinetic of a β-lactam antibiotic, amoxicillin, co-administered with pectin bead-entrapped β-lactamases in a rat model.

EXPERIMENTAL METHODS

Materials

Amidated low methoxylated (LM) pectin (Unipectine™ OG 175C, degree of esterification from 22% to 28% and degree of amidation from 19% to 23%) was a gift from Degussa Texturant Systems (Boulogne-Billancourt, France). Penicillinase (3175 I.U. of benzylpenicillin substrate per mg of protein) from *Bacillus cereus*, a mixture of β-lactamases I and II, was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Nitrocefin, a chromogenic cephalosporin used for the evaluation of β-lactamase activity, was provided by Oxoid (Basingstoke, England). Calcium chloride was obtained from Acros Organics (Geel, Belgium). Polyethylenimine (PEI, M_w 25 kDa, branched, water free), Hepes, EDTA, Pepsin, Pancreatin from porcine pancreas and Pectinex® SP-L (9.4 U/mL), a mixing of pectinases from *Aspergillus aculeatus*, were purchased from Sigma–Aldrich. Sodium chloride was obtained from VWR (Strasbourg, France).

Preparation of Pectin Beads

Calcium pectinate beads were prepared by ionotropic gelation. Briefly, a 6% (w/v) amidated pectin solution was obtained by dissolving 1.5 g of pectin OG 175C in 25 mL of distilled water. Penicillinase was then added to the pectin solution at the final concentration of 0.12 mg of protein/mL (385 U of benzylpenicillin/mL). This solution was dropped, using a peristaltic pump and a nozzle of 0.5 mm inner diameter, into a 6% (w/v) calcium chloride solution at room temperature, under stirring. By contact with calcium ions, pectin droplets instantaneously formed gelled beads. These beads were allowed to stand in the calcium chloride solution for 20 min and were then separated by filtration, washed by distilled water and dried at 37°C for 2 h. Beads obtained by this process are called "nude beads." To achieve coating, undried nude beads were introduced into an aqueous solution of PEI 0.6, 0.8, or 1% (w/v), during 20 min and then separated by filtration and dried for 2 h at 37°C. Beads prepared by this process are named "coated beads."

Characterization of Pectin Beads

Scanning Electron Microscopy (SEM)

Nude and coated beads loaded or not with β-lactamases, were covered with Platinum/Palladium under vacuum to a thickness of about 4 nm (Cressington 208 HR, Eloise, France). Morphological examination of the bead surface was