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Exploiting gastrointestinal bacteria to target drugs to the colon: An in vitro study using amylose coated tablets

Peter J Wilson, Abdul W Basit *

The School of Pharmacy, University of London, 29/39 Brunswick Square, London WCIN 1AX, UK

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Abstract

The bacterial substrate amorphous amylose, in the form of a film coating, provides a means of delivering drugs to the colon. This coating has traditionally been applied to multi-unit systems, in part because of the small size and divided nature of this type of dosage form, which provides a large surface area for enzymatic attack and drug release. The present study was conducted to explore the utility of the coating for colonic targeting of single unit tablet systems. Amylose was combined with the water-insoluble polymer ethylcellulose, which acts as a structuring agent, in different proportions to produce film coatings of various thicknesses for application to mesalazine (mesalamine or 5-aminosalicylic acid)-containing tablets. Drug release from the coated products was assessed under pH dissolution conditions resembling the stomach and small intestine, and also in conditions simulating the colon using a batch culture fermenter inoculated with human faecal bacteria. The rate and extent of drug release was related to the ratio of amylose to ethylcellulose in the film and the thickness of the coating. Increasing the proportion of ethylcellulose in the film and/or the thickness of the coating depressed the rate of drug release in the conditions of the upper gastrointestinal tract. Drug release from the coated products was accelerated in the fermentation environment of the colon. This is attributed to bacterial digestion of the amylose component of the film coat producing pores for drug diffusion. This work indicates that amylose coated tablet formulations are promising vehicles for drug delivery to the colon.

Keywords: Amylose; Starch; Mesalazine; Microflora; Colonic delivery; Film coating

1. Introduction

The gastrointestinal tract is home to a viable microflora, with more than 400 bacterial species usually present (Finegold et al., 1983). Although these bacteria are distributed throughout the gastrointestinal tract, the vast majority are found in the large intestine (or colon) where the bacterial count is 10^{11} cfu/ml, compared to 10^4 cfu/ml in the small intestine (Finegold et al., 1983; Cummings et al., 1989). Colonic bacteria are fundamentally anaerobic in nature and are involved in the fermentation of carbohydrates and proteins that

* Corresponding author. Tel.: +44 20 7753 5865; fax: +44 20 7753 5865.
E-mail address: abdul.basit@ulsop.ac.uk (A.W. Basit).

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have escaped digestion in the stomach and small intestine (Cummings et al., 1989).

The carbohydrate starch is an important energy reserve of plants. It is synthesised as microscopic granules in the tissues of many plant species, and has provided dietary energy for animals and man for several millennia. Starch is composed of two polysaccharides: amylose and amylopectin. Amylose is an essentially linear α-glucan containing α-(1,4) bonds. The molecular weight is approximately 1×10^5 to 1×10^6 (Biliaderis, 1998). Amylopectin has a much higher molecular weight than amylose, typically 1×10^7 to 1×10^9 and is much more heavily branched, with about 95% α-(1,4) and 5% α-(l,6) bonds (Biliaderis, 1998). The amount of amylose usually present in starch is between 20 and 35%, although breeders have managed to develop starches, which contain no amylose or those, which contain between 50 and 80% (Biliaderis, 1991). For nutritional purposes, dietary starch has been classified into three main types, rapidly digestible starch, slowly digestible starch and resistant starch (Englyst and Hudson, 1996). Resistant starch can be further subdivided into four types, physically inaccessible starch, resistant starch granules, retrograded starch and chemically modified starch (Haralampu, 2000). In vivo, all four types of resistant starch resist digestion in the small intestine and become available for fermentation in the colon.

One form of starch, amylose, can be made resistant to pancreatic enzymes through the formation of an amorphous structure (amorphous amylose), and can be degraded by colonic bacteria (Miles et al., 1985; Ellis and Ring, 1985). This form of amylose has been utilised as a carrier for drug delivery to the colon (Milojevic et al., 1996). Targeting drugs to the colon has major implications in a number of disorders, including the direct treatment of conditions such as ulcerative colitis, Crohn's disease, irritable bowel syndrome and colonic carcinoma (Basit, 2005). Amylose has the ability to form films through gelation, which in combination with the water-insoluble polymer ethylcellulose provides the basis for film coatings suitable for application to solid dosage forms (Milojevic et al., 1996; Siew et al., 2000a; Leong et al., 2002). These coatings have routinely been applied to multi-unit systems such as pellets. By virtue of their small size and divided properties, coated pellets provide a large surface area for enzymatic attack, which should lead to rapid and consistent drug release. This has been confirmed by a number of studies both in vitro and in vivo (Milojevic et al., 1996; Cummings et al., 1996; Siew et al., 2000b, 2004; Basit et al., 2004). Single-unit systems provide an alternative and more common platform for oral modified-release drug delivery, primarily because of their ease and cost of manufacture. Limited data is available on the performance of the amylose-based coating on single-unit systems (Tuleu et al., 2002). The present study therefore was conducted to assess the drug release characteristics of amylose coated tablets under simulated gastric, small intestinal and colonic conditions.

2. Materials and methods

2.1. Raw materials

The model drug mesalazine (5-aminosalicylic acid) was obtained from Sigma–Aldrich, Poole, UK, lactose was purchased from Sheffield Products, Norwich, USA, and polyvinyl pyrrolidone (MW 44,000) and magnesium stearate were sourced from VWR Chemicals, Poole, UK. The Institute of Food Research, Norwich, UK supplied the amylose, previously extracted from pea starch, in the form of an amylose–butan-1-ol complex aqueous dispersion. The amylose was used at a concentration of 12% (w/w). Ethylcellulose N-100 was obtained from Dow Chemical Co. Ltd., Uxbridge, UK. The solvent ethanol and plasticiser dibutyl sebacate were purchased from Sigma–Aldrich, Poole, UK. Pepsin (1:2500 potency) and pancreatin (potency equivalent to USP specification) were purchased from Sigma–Aldrich, Poole, UK. All other chemicals were of AnalaR grade and were obtained from VWR, Poole, UK.

2.2. Fabrication of mesalazine tablets

Tablets were prepared by wet granulation to the following formula: 15% mesalazine, 79% lactose, 5% polyvinyl pyrrolidone and 1% magnesium stearate. The tablets were manufactured using a single punch tabletting machine (Manesty, Speke, UK). The tablets were bi-convex in design, 8 mm in diameter and 200 mg in mass. The dose of mesalazine in each tablet was 30 mg.

2.3. Preparation of amylose–ethylcellulose film coating systems

Ethylcellulose was dissolved in ethanol to produce a 3% (w/v) solution. The plasticiser dibutyl sebacate was added to the solution at a concentration of 20% (w/w) based on the solid dry weight of ethylcellulose present, and mixed for 3 h using a magnetic stirrer. Various quantities of the amylose–butan-1-ol complex aqueous dispersion were added to the plasticised ethylcellulose solutions and stirred for a further 1 h to produce coating formulations with different solid ratios of amylose to ethylcellulose (1:1, 1:2 and 1:3).

2.4. Film coating of mesalazine tablets

The tablets were coated using Strea-1 bottom spray fluidised bed coater (Aeromatic AG, Bubendorf, Switzerland). The coating procedure involved maintaining the inlet temperature at 45 °C, spray rate at 1 g/min and atomising pressure at 0.2 bars. Fifty grams batches of tablets were coated each time. A series of coated products were produced with different film thicknessess (expressed as the % total weight gain, %TWG), ranging from 2 to 6%.

2.5. Dissolution studies

Mesalazine release from the film-coated tablets was assessed by dissolution testing using an USP type II paddle dissolution apparatus (model PTWS, Pharma Test, Hainburg, Germany). The tests were performed using a paddle rotation speed of 50 rpm in 900 ml dissolution medium at 37.0 °C. The pH and nature of the dissolution medium were varied over the course of the experiment: pH 1 hydrochloric acid with and without 0.32% pepsin for 3 h (to resemble the conditions in the stomach), followed by pH 7.2 phosphate buffer with and without 1% pancreatin for 3 h (to simulate the conditions of the small intestine). The quantity of drug released from the dosage form was investigated at regular intervals by an in-line UV spectrophotometer. Each experiment was run in triplicate. The results are expressed as cumulative percentage drug release versus time profiles.

2.6. Fermentation studies

Mesalazine release from the film coated tablets was also assessed in conditions simulating the human colon. One tablet was introduced into individual 100 ml batch culture fermenters inoculated with human faeces (10%, w/v). The fermenters were prepared by homogenising freshly voided human faeces from three healthy subjects in a phosphate-based buffer medium of pH 7.2 (Silvester et al., 1995; Basit et al., 2002). The fermenters were sealed under positive nitrogen pressure to establish an anaerobic environment and then placed in an incubator at 37 °C and shaken at 100 rpm. Control experiments, buffer medium without the presence of faeces, were also run in parallel. Each experiment was run in triplicate. Two millilitre samples were removed at hourly intervals over a 6-h period, centrifuged at 13,000 rpm for 5 min and then filtered through 0.2 μm filters prior to analysis for drug concentration by HPLC (series 400, Perkin-Elmer, Norwalk, USA) using a validated method (Siew et al., 2000b). The results are expressed as cumulative percentage drug release versus time profiles.

3. Results and discussion

The objective of the present study was to establish the utility of the amylose–ethylcellulose film coating system for colonic delivery of single-unit tablet systems. To this end, mesalazine-containing tablets were coated with three different amylose–ethylcellulose formulations and subsequently assessed under simulated conditions of the gastrointestinal tract. Each coating differed in terms of the proportion of amylose to ethylcellulose in the formulation (amylose:ethylcellulose ratio, 1:1, 1:2, 1:3). Each formulation was also coated to different film thicknesses (TWG of 2, 4 and 6%). The rate of drug release from these three formulations under pH conditions mimicking the stomach and small intestine are shown in Figs. 1–3. It is evident that the rate of release is inversely proportional to the thickness of the coat, suggesting that the film coat is controlling the release process. The rate of release is also influenced by the quantity of amylose present in the film. These mixed amylose and ethylcellulose formulations produce porous heterogeneous film structures (Siew et al., 2000a; Leong et al., 2002). In an aqueous

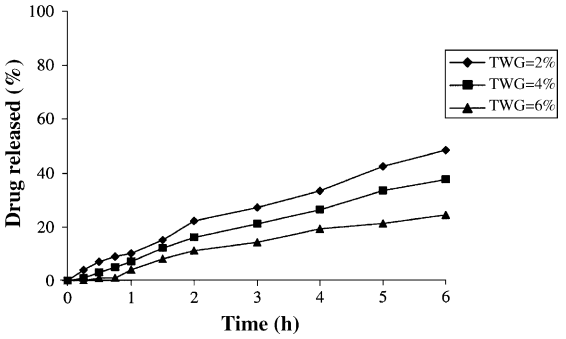

Fig. 1. Influence of coat thickness (%TWG) on mesalazine release from tablets coated with a mixture of one part amylose and one part ethylcellulose under simulated gastric and small intestinal conditions.

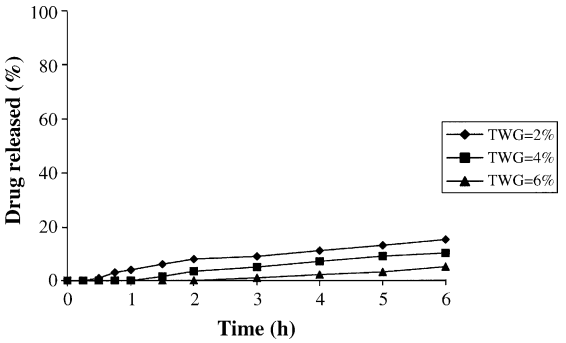

Fig. 2. Influence of coat thickness (%TWG) on mesalazine release from tablets coated with a mixture one part amylose and two parts ethylcellulose under simulated gastric and small intestinal conditions.

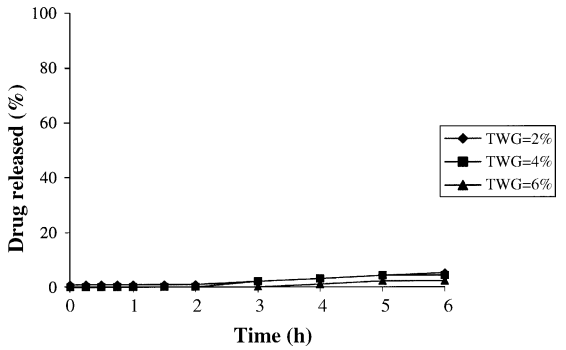

Fig. 3. Influence of coat thickness (%TWG) on mesalazine release from tablets coated with a mixture one part amylose and three parts ethylcellulose under simulated gastric and small intestinal conditions.

environment, akin to the conditions of the dissolution test or the gastrointestinal tract, amylose swells and disrupts the organisation of the film coat, leading to the formation of aqueous filled pores. These pores provide a channel for drug diffusion from the core of the tablet to the external environment. This route of release is in addition to drug release through the plasticised amylose and ethylcellulose components of the film. It is clear from Figs. 1–3 that film coatings containing higher proportions of amylose are more permeable to drug release.

In effect, the amylose is acting as a pore-forming agent within these ethylcellulose film structures. The addition of pore-forming agents, such as hydroxypropyl methylcellulose, to water-insoluble films is a common approach to increase coat permeability and drug release from extended release products (Yuen et al., 1993; Frohoff-Hulsmann et al., 1999; Chan et al., 2005). However, in the context of colonic delivery pore formation and film permeability must be retarded in the upper gastrointestinal tract to minimise premature drug release. Since mouth to colon transit time is normally of the order of 6 h (Christensen et al., 1985; Clarke et al., 1993; Abrahamsson et al., 1996), the 6-h dissolution test used in these studies provides a means to screen potential formulations for resistance. Tablets coated with one part amylose and one part ethylcellulose are freely permeable and would release much of their drug load before reaching the colon (Fig. 1). However, the formulations containing lower levels of amylose are more resilient and should provide the necessary barrier properties (Figs. 2 and 3).

The tablets coated with 1 part amylose and 2/3 parts ethylcellulose were further tested for resistance under physiological conditions more closely resembling those of the stomach and small intestine. The presence of pepsin and pancreatic enzymes had no effect on drug release (data not shown). This lack of effect highlights the resistance of the coatings to enzymatic degradation in the upper gastrointestinal tract. This also confirms that the physical form of amylose in the film coating is amorphous amylose.

Further investigations were conducted using a batch culture fermentation system inoculated with human faecal bacteria. This system provides the basis for an in vitro model of the human colon. Fig. 4 highlights the drug release characteristics of the one part amylose and two parts ethylcellulose film coated tablets.

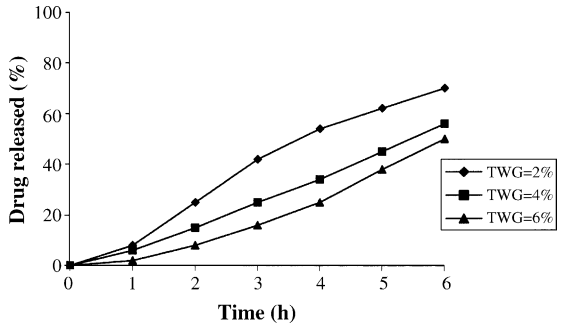

Fig. 4. Influence of coat thickness on mesalazine release from tablets coated with a mixture of one part amylose and two parts ethylcellulose under simulated colonic conditions of the fermentation system.

Release occurs from all the coated formulations. As expected, the rate of mesalazine release is related to the thickness of the coating, with the tablet coated with the thinnest film releasing at the fasted rate. In contrast, the rate of release in the control was appreciably slower, with less than 15% drug release at 6 h for all the formulations (data not shown). This indicates that the bacterial enzymes within the fermenter are digesting the film coat. Ethylcellulose is recalcitrant to bacterial action (Siew et al., 2000a; Leong et al., 2002), hence it is the amylose component of the film that is being acted upon. Bacterial digestion of the amylose fraction generates pores in the film structure through which the drug diffuses to the external medium. Similar digestion results were obtained with the one part amylose and three parts ethylcellulose coatings (data not shown), although the rate of release was slower presumably because of the reduced percentage of amylose in the coat.

4. Conclusions

This study has shown that it is feasible to exploit gastrointestinal bacteria to trigger mesalazine release from amylose-based systems. The data indicate that the ratio of amylose to ethylcellulose in the film and the thickness of the coating are the key parameters in controlling drug release from the system. This is also the first study to highlight the utility of the coating system for colonic delivery of single-unit tablet formulations, which in turn further broadens the technology's versatility.

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