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Design and evaluation of a novel chitosan-based system for colon-specific drug delivery

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ABSTRACT

Tritrichomonas foetus is a flagellated protozoan parasite that colonizes the feline colon causing colitis and chronic foul smelling diarrhoea. Despite the efficacy of Ronidazole in the treatment of *T. foetus*, Ronidazole has been reported to cause neurotoxicity in some cats due to rapid absorption in the small intestine. A novel amphoteric derivative of chitosan was synthesised and characterized. A combination of time, pH, and an enzyme controlled system was used in a study of a new compression coated tablet for delivery of Ronidazole to the colon. Axial, radial swelling and erosion of selected tablets were carried out in various media. The effect of weight ratio, enzyme and pH on *in vitro* drug release profile was investigated. The results show that less than 2% of the drug was released in the physiological environment of the stomach and small intestine.

1. Introduction

The treatment of gastrointestinal tract diseases has the advantage that orally administered drugs should present in much higher concentrations in the gastrointestinal tract (GIT) than those administered *via* systemic circulation. However, absorption of drugs in the proximal intestine may prevent therapeutic concentrations more distally. In other words, drug delivery to the various regions of the GIT must be tailored accordingly. This is especially true of colonic diseases. *Tritrichomonas foetus* (*T. foetus)* is a flagellated protozoan parasite that colonizes the feline colon and distal ileum, causing colitis and chronic foul smelling diarrhoea [1–4]. Ronidazole (RDZ) is currently the most widely used drug to treat *T. foetus* intestinal infection in cats [2,5,6]. RDZ is a nitroimidazole which is reduced by anaerobic organisms such as *T. foetus* to autotoxic free radicals and causes destabilization of the organism's DNA and subsequent death [2,7]. Despite its efficacy in the treatment of *T. foetus*, RDZ has been reported to cause neurotoxicity in some cats due to rapid absorption in the small intestine which is thought to cause high plasma concentrations of the drug [2,5]. Therefore delaying systemic absorption until colonic delivery would be hugely beneficial. Polysaccharides have gained much attention in developing colon specific drug release systems because of their flexibility in obtaining a desirable drug release profile, cost effectiveness, ease of modification, biocompatibility, biodegradability and ability to form hydrogels [8,9].

Among the various polysaccharides, chitosan (Cts) has attracted significant attention for the design and development of colon targeted delivery systems due to its potential degradation by the enzymes present in the colon [10–16]. Chitosan is a hydrophilic polyelectrolyte polysaccharide composed of glucosamine and *N*-acetyl glucosamine units linked by β(1–4) glycosidic bonds, produced by alkaline *N*-deacetylation of chitin [17].

A survey of literature reveals that, guar gum-coated tablets are the only polysaccharide based tablet used for targeted colon release in cats [18].

In this present study, a novel amic acid derivative of Cts was synthesised and characterized. Due to the importance of amphoteric polymers coupled with the low price of pyromellitic dianhydride (PMDA) and the facile preparation method as compared to other amphoteric Cts based biomaterials, this new amphoteric polymer should attract considerable scientific and commercial interest.

We now report the development and evaluation of a novel colon targeting drug delivery system using a combination of Cts and CPAA as a new compression coating for RDZ.

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2. Materials and methods

2.1. Materials

Chitosan was purchased from Acros Organics (Geel, Belgium). Pyromellitic dianhydride (PMDA), Ronidazole (RDZ), trypsin from porcine pancreas, α-chymotrypsin from bovine pancreas, pepsin, pancreatin and β-glucosidase from almonds were all obtained from Sigma–Aldrich (Auckland, New Zealand).

2.2. Preparation of Cts-PMDA amic acid (CPAA)

Chitosan (2.00 g, 0.0124 mol of glucosamine residues) and glacial acetic acid (50 mL) were added to a 200 mL round bottomed flask. The mixture was stirred at room temperature for 1 h. Pyromellitic dianhydride (2.71 g, 0.0124 mol), completely dissolved in DMF (100 mL) was added to the flask. The mixture was stirred for 24 h at room temperature. The crosslinked hydrogel that formed was filtered off, washed with DMF and methanol and then freeze dried.

2.3. Characterization of the hydrogel

The FTIR spectra of the matrix were recorded using a Nicolet 5700 FTIR spectrometer in the range of 4000–400 cm^{-1}. Solid-state carbon-13 (^{13}C) magic angle spinning (MAS) NMR spectra were obtained at a ^{13}C frequency of 50.3 MHz on a Bruker (Rheinstetten, Germany) DRX 200 MHz spectrometer. The X-ray diffraction patterns were recorded on a Rigaku SPIDER curved image-plate detector using a multi-metal-layer Osmic confocal optic to monochromate and focus the Cu-Kα radiation produced by a Rigaku MM007 micro-focus rotating-anode generator. The surface morphology of the powders was studied using an FEI Quanta 200 Scanning Electron Microscope (Eindhoven, The Netherlands) at an accelerating voltage of 20 kV. Elemental analysis was performed using a Carlo Erba Elemental Analyser EA 1108 using a flash combustion technique (Campbell Microanalytical Laboratory, Otago University, Dunedin, New Zealand). A TA Instruments SDT Q600 instrument was used for simultaneous DTG and TGA data acquisition. Data were analysed using TA Universal Analysis software. Samples were loaded into an aluminium oxide crucible and heated at a rate of 5 °C/min to 600 °C under a dynamic nitrogen atmosphere.

2.4. Enzyme inhibitory effect

2.4.1. Trypsin inhibition study

The enzymatic inhibitory activity of Cts and CPAA matrices towards trypsin was evaluated with *N*-α-benzoyl-L-arginine *p*-nitroanilide (BAPNA) as the substrate [19]. Polymer matrices were dispersed in Tris-buffer (1 mL, 0.05 M, pH 8.2) containing $CaCl_2$ (0.02 M) to reach a final concentration of 0.1% w/v solutions. After adding 30 U of trypsin solution (0.3 mL in 10 mM HCl), the mixture was incubated at 37 °C. Thereafter, 2.1 mL of the BAPNA solution (20 mM in DMF) was added and incubation continued for 15 min at 37 °C. After stopping the enzymatic action with 1% trichloroacetic acid solution, the nitroaniline formed was analysed by measuring the absorbance at 405 nm using a UV/visible spectrometer.

2.4.2. α-Chymotrypsin inhibition study

The assay of α-chymotrypsin inhibition by Cts and CPAA was performed with *N*-benzoyl-L-tyrosine ethyl ester (BTEE) solution as the substrate [20]. Polymer matrices were dispersed in Tris–HCl buffer (1 mL, pH 7.8) containing $CaCl_2$ (0.02 M) to get a final concentration of 0.1% w/v solutions. After 15 min incubation in 25 °C, chymotrypsin solution (0.3 mL, 2 mg/mL in 1 mM HCl) was added. Thereafter, BTEE (1.4 mL, 37 mg dissolved in 63 mL of methanol and 37 mL of demineralized water) was added and the mixture was incubated at 37 °C for 5 min. After stopping the enzymatic action with 1% trichloroacetic acid solution, absorbance at 256 nm (resulting from the hydrolysis of benzoyl-L-tyrosine ethyl ester) was recorded.

2.5. Cell cultures

Crandall Rees feline kidney (CRFK) cells were maintained in an advanced Dulbecco modified medium (Adv DMEM, Invitrogen) supplemented with 2% (growth medium) or 1% (maintenance medium) fetal bovine serum (Thermo Scientific, MyClone), 1% antibiotic solution to a final concentration of 100 units/mL of penicillin and 100 μg/mL of streptomycin (PenStrep, Invitrogen), and 1% glutamax (Invitrogen). The cells were seeded into pre-selected wells of 96-well tissue culture plates (Thermo Scientific, Nunc) at a concentration of 5×10^4 cells/well in a 100 μL volume. The plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2 overnight.

2.6. Cytotoxicity test

WST-1 assay was carried out to test the cytotoxicity of Cts and CPAA. The compound suspensions (10 mg/mL) were subjected to serial 2-fold dilutions in the maintenance medium. The growth medium was removed from pre-seeded 96-well plates after approximately 24 h, when the monolayers were 95% confluent, and replaced with 200 μL of each compound's dilution, from 0.62 mg/mL to 0.005 mg/mL in duplicate (test wells). For each compound dilution, an equal amount (200 μL) was also added in duplicate to wells that were not pre-seeded with cells (control wells). The growth medium was similarly replaced in 4 cell control wells (pre-seeded with cells, without addition of any compounds) and added to 4 medium control wells (empty wells not pre-seeded with cells). Plates were incubated at 37 °C, 5% CO_2 humidified atmosphere for 24 h. The cultures were inspected visually for signs of toxicity using an inverted microscope. In addition, the number of viable cells in each well was quantified using a colorimetric cell viability assay (WST-1 reagent, Roche), according to the manufacturer's instructions. The % cell viability as compared to control cells was calculated separately for each dilution of each compound based on the optical density readings measured at 450 nm wavelength following a 2.5 h incubation of cells in the presence of the WST-1 reagent.

2.7. Preparation and evaluation of tablets

2.7.1. Preparation of compression-coated tablets

Cts:CPAA dry blends with a total weight of 400 mg at various % weight ratios were used to achieve compress-coated tablets with a 100 mg core (80 mg drug and 20 mg α-lactose monohydrate) at a compression force of 50 kN using a hydraulic press and a 13-mm diameter die and flat-face punch set.

2.7.2. Tablet crushing strength

The thickness (T) and diameter (D) of the matrix tablets was determined using a caliper (Mitutoyo Dial Thickness Gauge, Mitutoyo, Japan). A TA.XT Plus Texture Analyzer (Stable Micro Systems, UK) was used to measure the hardness of tablets. The tablet tensile strength is the force required to break a tablet by compressing it in the radial direction. A 5-mm-diameter spherical puncturing probe was driven through the tablet with a speed of 1 mm/min. The load