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Drug delivery to inflamed colon by nanoparticles: Comparison of different strategies

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ABSTRACT

For inflammatory bowel disease (IBD) treatment, local delivery of molecules loaded in nanoparticles to the inflamed colon could be a promising strategy. The aim of this study was to investigate how drug-loaded polymeric nanoparticles target the site of inflammation and to analyse the influence of different colon-specific delivery strategies. Three different polymeric nanoparticles were formulated using ovalbumin (OVA) as a model drug. pH-sensitive nanoparticles were made with Eudragit® S100. Mucoadhesive nanoparticles were created with trimethylchitosan (TMC). A mix of polymers, PLGA, PEG-PLGA and PEG-PCL, were used to obtain a sustained drug delivery. Furthermore, ligands targeting immune cells (i.e. mannose) or the inflamed colon (i.e. a specific peptide) were grafted on the PEG chain of PCL. Interaction of nanoparticles with the intestinal epithelium was explored using Caco-2 monolayers designed to mimic an inflamed epithelium and then visualized using confocal laser microscopy. TMC nanoparticles had the highest apparent permeability for OVA in the untreated model. However, in the inflamed model, there were no difference between TMC, PLGA-based and Eudragit® nanoparticles. The uptake of nanoparticles in the inflamed mouse colon was assessed in a horizontal diffusion chamber. Mannose-grafted PLGA nanoparticles showed the highest accumulation of OVA in inflamed colon. Based on these results, active targeting of macrophages and dendritic cells may be a promising approach for targeting the colon in IBD.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD), two of the major inflammatory bowel diseases (IBD), are relapsing and chronic inflammatory disorders of the intestinal mucosa, with an organ-susceptibility for the colon (Carter et al., 2004). Conventional therapy has been extensively used during five decades to treat IBD with, however, serious adverse effects (Grimpen and Pavli, 2010; Rogler, 2010). Thus, in the last 15 years, alternative biological therapies have been developed to treat IBD. In this context, tumour necrosis factor alpha (TNFα) antibodies have been widely used with success. However, these biopharmaceutics are administered systemically and several side effects and complications are associated with their use (Plevy and Targan, 2011; Stallmach et al., 2010).

A targeted colonic carrier of these biopharmaceutical drugs could be a major advance to treat such diseases by delivering the drug locally in the colon and reaching the specific site of inflammation. Different strategies have been used in the literature to target the inflamed colon after oral administration. Pro-drugs, exploitation of higher colonic pH values, enzymatic activity of colonic microflora, intestinal transit time or colonic pressure have been investigated as specific colonic drug delivery strategies the last decades (Friend, 2005; Meissner and Lamprecht, 2008). However, the overall dimensions of the delivery system significantly influence the targeting of the inflamed colon. Size-dependency of particles impacts their epithelial uptake and a preferential accumulation in the inflamed colon has been found for 100 nm-sized particles. Indeed, it has been demonstrated in experimental colitis that nanosized particles are taken up more readily by immune-related cells such as macrophages or dendritic cells in the area of active inflammation. They can also better attach to mucus layers due to their easier penetration and their relatively small mass (Lamprecht et al., 2001). Furthermore, it seems that several pathophysiological changes due to mucosal inflammation are involved

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in this preferential uptake: (i) disrupted intestinal barrier due to the presence of mucosal surface alterations, crypt distortions and ulcers, (ii) infiltration of immune related cells as macrophages, lymphocytes or dendritic cells and (iii) higher mucus production (Cornaggia et al., 2011; Moulari et al., 2008). This accumulation of nano-sized drug delivery systems should locally deliver higher amounts of entrapped drugs to the inflamed areas, thus leading to a better therapeutic efficacy and a decrease in systemic side effects.

An initial approach to target inflamed areas of the colon was to combine specific colonic drug delivery strategies with nanocarriers. Polymeric nanoparticles were chosen as carriers able to fulfil such criteria. Based on the literature, three colonic delivery strategies for nanocarriers were selected. In the gastrointestinal tract, from the stomach to the colon, intraluminal pH varies progressively up to 7. In order to obtain pH-sensitive nanoparticles, Eudragit® S100, a polymer of methacrylic acid and methyl methacrylate, which dissolves when the pH is above 7, was used to release the entrapped drug (Damge et al., 2010; Makhlof et al., 2009). N,N,N-trimethylchitosan chloride (TMC) was used to prepare mucoadhesive nanoparticles. TMC, which is the result of the partial quaternization of chitosan, is characterized by fixed positive charges irrespective of pH, making it soluble at a colonic pH (>7), unlike chitosan. Similar to chitosan, TMC opens tight junctions between epithelial cells, leading to an increase of paracellular permeability. It also has a special characteristic to adhere to the mucosal surface because this cationic polymer is attracted by negatively charged intestinal mucosa (Plapied et al., 2010). Furthermore, chitosan and its derivatives are not degraded in the upper gastrointestinal tract, but undergo enzymatic hydrolysis by colonic microbiota (Jain et al., 2007; Mourya and Inamdar, 2009). Biodegradable nanoparticles were made using a mix of three polymers: poly(lactic-co-glycolic) acid (PLGA), poly(lactic-co-glycolic) acid-block-polyethyleneglycol (PLGA-PEG) and poly-ε-caprolactone-block-polyethyleneglycol (PCL-PEG) to get sustained release of the entrapped drug (Fredenberg et al., 2011) and to compare active from passive targeting by the grafting of specific ligands on the PEG (Garinot et al., 2007). PLGA nanoparticles have been shown to accumulate in ulcerous lesions of IBD in human (Schmidt et al., 2010). A peptidic sequence identified by phage display as well as a mannose derivative were selected as ligands, to target the inflamed colon and immune-related cells respectively (Fievez et al., 2009; Takagi et al., 2007).

The aim of this study was to compare different strategies to locally deliver biopharmaceutical drugs to inflamed colonic tissue using oral administration of nanoparticles. Therefore, a model protein, ovalbumin (OVA) was encapsulated in (i) bioadhesive TMC nanoparticles, (ii) pH-sensitive nanoparticles made with Eudragit® S100 and (iii) PLGA-based nanoparticles to obtain a sustained delivery. The features of these different nanoparticles including size, zeta potential, encapsulation efficiency and drug loading were examined. The epithelial cell viability upon exposure to the nanoparticles was determined. The transepithelial transport of the different nanoparticles was investigated using Caco-2 monolayers mimicking the inflamed colon and visualized using confocal laser microscopy. In an inflamed colonic murine tissue, the uptake of these different nanoparticles was evaluated in horizontal diffusion chambers ex vivo.

2. Materials and methods

2.1. Materials

2.1.1. Cell lines

Human colon carcinoma Caco-2 line (clone 1), obtained from Dr. Maria Rescigno, University of Milano-Bicocca, Milano, Italy, was used (des Rieux et al., 2007a,b).

2.1.2. Cell culture

Dulbecco's modified Eagle minimal essential medium (DMEM, 4.5 g/l D-glucose), non-essential amino acids, L-glutamine and penicillin–streptomycin (PenStrep) were purchased from Gibco™ Invitrogen Corporation (Paisley, UK). HyClone® foetal bovine serum (FBS) from South America was purchased from Thermo Fisher Scientific (Waltham, MA). Trypsin 0.05% with EDTA was purchased from Gibco™ Invitrogen Corporation. Dulbecco's PBS without $CaCl_2$ and $MgCl_2$ (D-PBS) and Hank's Balanced Salt Solution (HBSS) were purchased from Gibco™ Invitrogen Corporation.

Rhodamine-phalloidin was obtained from Molecular Probes (Eugene, OR). UltraCruz mounting medium was obtained from Santa Cruz (Delaware, CA). Triton X-100, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dimethyl sulphoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO). Transwell® polyethylene terephthalate (PET) filters were purchased from Corning (Lowell, MA). Inserts were coated with Matrigel™ Basement Membrane Matrix (Becton Dickinson, Franklin Lakes, NJ) before cells seeding. IL-1β, TNF-α and LPS were obtained from Sigma–Aldrich. IFN-γ was purchased from Chemicon-Millipore (Billerica, MA).

2.1.3. Chemicals

N,N,N-trimethylchitosan chloride (TMC) (degree of acetylation: 22.3 mol%; degree of quaternization: 33 mol%) was a gift from KitoZyme (Herstal, Belgium). Pentasodium tripolyphosphate (TPP), dichloromethane, ovalbumin (OVA) grade V, polyvinyl alcohol (PVA average 13,000–23,000) and sodium cholate were obtained from Sigma–Aldrich. FITC-ovalbumin (FITC-OVA) was purchased from Molecular Probes (Eugene, OR). Eudragit® S100 was a gift from Evonik RÖHM GmbH (Darmstadt, DE). Poly(lactic-co-glycolic) acid (PLGA) (L:G 50:50, average Mw 7000–17,000) was obtained from Boehringer Ingelheim GmbH (Ingelheim am Rhein, DE). PEGylated poly(lactic-co-glycolic) acid (PLGA-PEG average Mw 16,500–4600) was synthesized by ring opening polymerization as previously described (Vangeyte et al., 2004; Zweers et al., 2004). PCL-PEG (12,000–6000 g/mol) was synthesized. The molecular clip (i.e. O-succinimidyl-4-(1-azi-2,2,2-trifluoroethyl) benzoate) was synthesized as described elsewhere (Pourcelle et al., 2012).

2.1.4. Radio-labelling of ovalbumin

Sodium boro[^{3}H]hydride (100 mCi) was supplied by Perkin Elmer (Boston, MA). Radiolabelling of OVA (Sigma) was performed by reductive alkylation of amino groups (Means and Feeney, 1968). The [^{3}H]-ovalbumin specific activity was obtained by measuring the total protein concentration by a microBCA protein assay Kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions, and the radioactivity by liquid scintillation. The protein integrity after labelling was evaluated by SDS-PAGE and ELISA analysis according to standards protocols (data not shown) (Garinot et al., 2007).

2.2. Ligands

The ligands grafted on PCL-PEG were the following:

- Gly-Ser-Gln-Ser-His-Pro-Arg-His (GSQSHPRH) hexapeptide, identified by phage display as specific to murine inflamed colon (Takagi et al., 2007), was purchased from Eurogentec (Seraing, Belgium).
- Man: Mannose derivative, i.e. 2-aminoethyl-α-D-mannopyroside, was synthesized as previously described (Pourcelle et al., 2009).