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Research paper

Colonic delivery of carboxyfluorescein by pH-sensitive microspheres in experimental colitis

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ABSTRACT

The colonic drug delivery in inflammatory bowel disease (IBD) by microcarriers has been suggested over the past decade; however, pharmacokinetic and biopharmaceutical details are hardly known. A model colitis was induced to male Wistar rats by trinitrobenzenesulfonic acid. Carboxyfluorescein (CF) was entrapped into microspheres (MS) prepared with the pH-sensitive polymer Eudragit® S100, in order to simulate drug delivery to the colon. Pharmacokinetic behaviour of CF–MS was compared to oral or rectal administration of CF as solution in healthy or colitis group. Colitis lowered the oral bioavailability of CF solution, compared to healthy controls (healthy: 8.4 ± 1.5; colitis: 3.0 ± 0.9; all μg/ml h), and similar results were obtained after rectal administration of CF solution (healthy: 5.6 ± 2.1; colitis: 1.8 ± 0.8). Surprisingly, CF–MS showed only minor differences between colitis and healthy controls (healthy: 1.9 ± 0.8; colitis: 2.3 ± 0.4). In contrary, the intra-tissue concentrations of CF of the various formulations in colitis showed lower levels than the comparable healthy group after oral drug administration. Pharmacokinetic outcome was largely disease-dependent, while CF–MS confirmed their ability to local drug delivery.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) usually affect different areas of the gut. Mucosal inflammation is mainly limited to the distal regions of the colon in UC. Transmural chronic inflammation may involve all intestinal segments in CD with a usual main focus in the distal parts of the small intestine [1]. The natural course of both variants consists of quiescent phases that are interrupted by relapses [2].

The general principle of a pharmacological treatment in inflammatory bowel disease [IBD] is to induce remission of outbreaks and to prevent outbreaks during remission. A large diversity of drugs is of therapeutic interest, such as 5-aminosalicylic acid and glucocorticoids but also immune suppressive drugs in severer cases [3–6]. For such highly potent drugs, a specific and locally limited delivery is most desirable in order to limit drug availability towards non-inflamed tissue, which lowers the efficiency and risk distinct adverse effects. Therefore, several strategies to deliver drugs to the large intestine after oral administration have been developed over the past decades. For instance, enzymatically degradable carriers rely on the enzymatic activity of colonic bacteria similar to the mechanism of prodrugs. However, disease related variability in colonic flora mainly observed in CD can distinctly impede efficient drug delivery [7,8]. Others, among them most of the commercialised systems, are based on the change of the luminal pH during the gastrointestinal passage [9–12].

The pH-sensitive approaches have been mainly reported for coated solid dosage forms using a variety of different polymers, such as methacrylates and substituted polyvinyl acetate and cellulose derivates [13]. Methacrylate/methacryl acid polymers Eudragit® S, L, and FS dissolve in aqueous media in the range of pH 6–7, respectively, which may be equivalent to a drug release to the distal ileum and seemingly most appropriate for IBD therapy.

Gastrointestinal transit in IBD can vary from that in healthy state due to mucosal inflammation, and the changes in the normal mechanism of transit. The inflamed colon presents abnormalities in fluid and electrolyte absorption and secretion [14]. An additional common pathophysiological attribute of ulcerative colitis patients is diarrhoea influencing the transit of the drug delivery system.

Solid dosage forms, especially drug carrier systems with a size larger than 200 μm are strongly subjected to the diarrhoea symptoms. This results in a decreased gastrointestinal transit time and leading therefore to a distinct risk of inefficiency. Thus, the efficiency of drug delivery systems can be decreased due to both accelerated carrier elimination and reduced drug release time and drug availability from the delivery system [15,16].

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A size reduction of the drug carrier system might be an option in order to circumvent those problems, as it was proposed by using microspheres (MS) [17,18].

Due to the smaller size of the delivery system, not only streaming becomes less dramatic but also other factors such non-specific mucoadhesion play a more important role than it is the case of standard oral dosage forms. This can have a significant impact when MS designed for colonic delivery also show mucoadhesive properties [19].

However, there are surprisingly few details in the literature on the pharmacokinetics of these experimental MS, especially in the context of IBD. Similarly, only little is known about how drug absorption takes place across the inflamed barrier at the inflammation site and tissue permeability is potentially modified. Also, the actual tissue concentrations after different delivery routes are rarely addressed.

In order to elucidate the role of the drug delivery system in healthy or diseased state and to estimate the efficiency of the local drug tissue accumulation, healthy rats and those suffering from an experimental colitis underwent pharmacokinetic studies using various administration routes and formulations. Besides, the intra-tissue concentration following the different administration pathways was recorded.

2. Methods

2.1. Materials

Eudragit® S100 was a kind gift from Degussa/Roehm Pharma Polymers (Darmstadt, Germany). Carboxyfluorescein (CF) and trinitrobenzenesulfonic acid (TNBS) were purchased from Cooper, Melun, France and Sigma–Aldrich Chemie GmbH (Steinheim, Germany), respectively. All other chemicals were of analytical grade.

2.2. Preparation of microspheres

Two hundred milligrams Eudragit® S and 25 mg CF were dissolved in 8 ml acetone/ethanol mixture 3:2 containing 10 μl of 1 N HCl. This solution was poured into 40 ml of liquid paraffin containing 4% w/w Span 80, and an oil/oil emulsion was formed by stirring with a three-blade propeller at 800 rpm for 2 h. The emulsion was stirred under vacuum until solvents were removed. MS were collected by filtration, and washing steps were performed with 150 ml *n*-hexane before drying at atmospheric pressure. All preparation steps were performed under subdued light.

2.3. In vitro characterisation of microspheres

Particle size analyses of all MS batches were carried out by laser light diffraction (Mastersizer® X, Malvern Instruments, UK). MS were dispersed in 2 ml of an aqueous solution of Tween 80 (0.2%). Particle morphology was analysed by SEM. MS were fixed on supports with carbon–glue and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then observed with the scanning electron microscope (JEOL JSM-5600 Scanning Electron Microscope, Tokyo, Japan) at 24 kV.

Drug loading was determined by fluorescence spectrophotometry after dissolution of MS in phosphate buffer at pH 7.4 as described elsewhere [20]. The in vitro drug release was analysed as follows: drug loaded MS were suspended in 100 ml phosphate buffer systems of pH 1.2, 4.5, and 7.4, respectively. The dissolution medium was kept under stirring at 100 rpm. All the experiments were carried out at 37 °C for 4 h. Aliquots of the dissolution medium (1 ml) were withdrawn at predetermined time intervals and replaced by fresh buffer. Drug concentrations in the supernatant were directly analysed by fluorescence spectrophotometry. CF was determined with an F-3010 Fluorescence spectrophotometer (Hitachi, Tokyo, Japan) without any additional processing.

2.4. Animal treatment

All animal experiments were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy of Sciences, US). The TNBS rat model was chosen as well recognised experimental model [21] that allow induction of colitis at an exact location. This permitted administration of rectal drug in the form of an exclusively local delivery to the inflamed tissue.

Male Wistar rats (10 weeks; $n = 4$/group) were treated by the following procedure in order to induce the TNBS model colitis: after light narcotizing with ether, the rats were catheterised 8 cm intrarectal, and 500 μl of TNBS (140 mg/kg) in an ethanol/water mixture was applied. Control groups received a rectal installation of an ethanol/saline mixture without TNBS instead. For 48 h, the rats were housed without treatment to maintain the development of a full inflammatory bowel disease model.

In order to ensure pharmacokinetic studies under reproducible conditions, colitis activity was quantified with a clinical score assessing weight loss, stool consistency, and rectal bleeding as previously described elsewhere [22]. No weight loss was counted as 0 point, 1–5% as 1 point, 5–10% as 2 points, 10–20% as 3 points and >20% as 4 points. For stool consistency, 0 point was given for well-formed pellets, 2 points for pasty and semiformed stools that did not stick to the anus, and 4 points were given for liquid stools that stick to the anus. Bleeding was scored as 0 point for no blood, 2 points for positive finding, and 4 points for gross bleeding. The mean of these scores was forming the clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

The determination of the myeloperoxidase activity (MPO) in resected colonic tissue samples allowed quantifying the severity of the colitis as a reliable index for the infiltration of activated neutrophils into the inflamed tissue. Activities were analysed according to a standard method [23]. Briefly, distal colon specimen was minced in 1 ml of hexadecyltrimethylammonium bromide buffer (0.5% in 50 mM phosphate buffer) on ice and homogenised. The homogenate was sonicated for 10 s, freeze–thawed three times, and centrifuged at 10,000 rpm for 3 min. Supernatant (0.1 ml) was added to 0.167 mg/ml of o-dianisidine hydrochloride and 0.0005% hydrogen peroxide, and the change in absorbance at 460 nm was measured.

All rats were anaesthetised with isoflurane during intravenous injection, oral administration, and blood sampling. In the experiments of oral CF administration, rats received either MS formulations (dispersed in 0.5% carboxymethyl cellulose) or CF solution at a dose of 1 mg/kg. For rats receiving a rectal administration of CF, the dye solution was administered in analogue to the TNBS induction procedure described earlier.

Blood specimens of 0.5 ml were collected from the jugular vein sampling at different predetermined time points in tubes containing 20 μl of heparin (2500 I.U/ml) as anticoagulant and centrifuged at 10,000*g* for 5 min. The plasma samples were diluted with phosphate buffer (pH 7.4) and analysed for their CF content by fluorescence spectrophotometry as described earlier [24].

2.5. Tissue penetration

Experimental setup was adapted from a method described elsewhere [25]. Control tissue samples were taken from the healthy group. Inflamed or non-inflamed tissue samples were taken from the treated healthy or colitis group, where inflamed tissues were