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The targeting of surface modified silica nanoparticles to inflamed tissue in experimental colitis

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ABSTRACT

One aspect in the emerging field of nanomedicine is site specific drug delivery via nanoparticles. The use of nanoparticles allows for increased therapeutic efficiency with a lowered risk for and extent of adverse reactions resulting from systemic drug absorption. 5-Amino salicylic acid (5ASA) loaded silica nanoparticles (SiNP) are proposed here as drug delivery system for specific accumulation in inflamed colonic tissues allowing for selective medication delivery to such inflammation sites. The drug was covalently bound to SiNP by a four-step reaction process. In-vitro toxicity of modified SiNP was tested in appropriate cell culture systems, while targeting index and therapeutic efficiency were evaluated in a pre-existing colitis in mice. Particle diameter was around 140 nm after final surface modification. In-vitro drug release demonstrated significant drug retention inside the NP formulation. Toxicity of the different formulations was evaluated in-vitro cell culture exhibiting a lowered toxicity for 5ASA when bound to SiNP. In-vivo, oral SiNP were found to accumulate selectively in the inflamed tissues allowing for significant amounts of drug load. SiNP demonstrated their therapeutic potential by significantly lowering the therapeutically necessary drug dose when evaluating clinical activity score and myeloperoxidase activity (untreated control: 28.0 ± 5.0 U/mg; 5ASA-solution (100 mg/kg): 8.2 ± 3.4 U/mg 5ASA–SiNP (25 mg/kg): 5.2 ± 2.4 U/mg). SiNP allow to combine advantages from selective drug targeting and prodrugs appearing to be a promising therapeutic approach for clinical testing in the therapy of inflammatory bowel disease.

1. Introduction

The general principle of drug treatment in inflammatory bowel disease (IBD) is to induce remission of outbreaks and to prevent outbreaks during remission. First-line therapy for patients with IBD has centred on sulfasalazine for decades. The identification of mesalamine 5ASA as the active moiety responsible for the luminal anti-inflammatory effects of sulfasalazine, as well as patient allergy, intolerance, or unresponsiveness to this agent has led to the development of multiple 5ASA conjugates, each with its own specific delivery system [1]. It is thus of tremendous importance to deliver 5ASA locally in order to reduce influences by systemic drug absorption causing adverse effects and drug loss lowering the probability for a therapeutic success.

Although many efforts have been made for a higher specificity of drug release by designing new drug delivery devices [2,3], all marketed delivery systems still appear to be insufficiently selective [4]. This is due to the fact that the drug release mechanisms are based on physiological parameters which are not related to the inflammation and barely to its location. Drug delivery is usually either triggered by the luminal pH in the gastrointestinal tract, the enzymatic activity of colonic bacteria, or the transit time of the drug carrier normally initiating the drug release in the distal ileum. As the inflamed areas affect only limited sectors of the intestinal tissue and may vary from patient to patient, consequently, pronounced drug amounts are still delivered unintentionally to non-inflamed regions. This misdirected drug release can be hardly avoided by the current state-of-the-art drug delivery systems, thus remaining a general problem responsible for undesirable adverse effects and potentially even therapy failure.

Among several new therapeutic approaches, a strategy was proposed to target the strong cellular immune response occurring in the inflamed regions, i.e., in general, an increased presence of neutrophils, natural killer cells, mast cells, and regulatory T cells, which play an important role in the pathophysiology of

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inflammatory bowel disease [5,6]. It was proven that particle uptake into those immune-related cells or the disrupted intestinal barrier at ulcerated regions [7] allows the selective accumulation of the particulate carrier system in the targeted area.

The increased adhesion of small particulate drug carriers to the inflamed tissue in ulcerative colitis led to a new therapeutic concept allowing for specific drug targeting in this disease [8–11]. This approach is mainly based on two pathophysiological changes in the inflamed tissue, allowing the higher adhesion of the carriers in the inflamed tissue caused by elevated levels of mucus production and an intensified particle uptake inside the colitis tissue as a result of an enhanced permeability and the presence of a highly increased number of immune related cells. This accumulation phenomenon was observed to be particle size dependent with an increased adhesiveness for smaller particle diameters [7]. A recent study on the comparative efficiency of this new delivery strategy and a pH-sensitive approach revealed a major drawback of the new system, namely the drug leakage during the intestinal passage of nanoparticles [12]. An early uncontrolled release of the active molecule reduces the therapeutic drug efficiency and may give rise to adverse effects. Due to the diffusion out of the small particles related to the enormous surface, the encapsulated drug is usually leaking easily towards the surrounding aqueous phase [13]. Subsequently, the probability that the drug molecules reach the colon is limited, especially since this leakage phenomenon is further enhanced for hydrophilic drugs such as 5ASA.

The use of silica nanoparticles (SiNP) in the biomedical field has been progressing for several years with a main focus on cell recognition [14] for diagnostic purposes as well as drug and gene delivery [15–17]. In cases where SiNP were utilized as medication carriers, drugs were adsorbed onto the surfaces of the NP. The drug release mechanism was triggered by simple desorption kinetics, which are poorly controllable in complex biological liquids (e.g. blood, lymph, and gastrointestinal juices). One major advantage of SiNP is that they are deemed toxicologically safe, which they have proven in 50 years of use as a pharmaceutical excipient for oral drug delivery.

We report here on the design of SiNP with modified surface for selective drug delivery towards inflamed tissue in chronic inflammatory diseases of the intestine. To date, ordinary nanoscale drug delivery systems tend to prematurely release drug compounds that are physically entrapped or adsorbed. As a solution to that problem, we propose here to bind the anti-inflammatory drug covalently to the surface of SiNP. The resulting chemical bond is biodegradable and intended to considerably delay drug release when compared to conventional physicochemical encapsulation. Minimizing unintended drug release during intestinal passage ensures targeted drug delivery to the site of action.

2. Methods

2.1. Drug–SiNP coupling

The first step was protective esterification of 5ASA. To a solution of anhydrous methanol (83.4 ml), containing 5ASA (0.033 mol) and cooled at 0 °C, thionyl chloride (7.7 ml) was added slowly. The mixture was stirred at room temperature for 1 h. The reaction mixture was then heated under reflux for 4 h. Afterwards solvent was removed under reduced pressure. The resulting precipitate was dissolved in ether and the solution was filtered. The filtrate was dissolved in a 1:1 (v/v) water/ether mixture at a pH of 9 prior to several washing steps with water and ether followed by a drying step with sodium sulphate.

Silica nanoparticles (3 g) were dispersed in toluene (100 ml). 3-Aminopropyltrimethoxysilane (10 ml) was added to the mixture under stirring and refluxed for 12 h. The resulting particles (2.85 g) were purified and dispersed in acetonitrile (100 ml) containing succinic anhydride (10 g). The solution was stirred and refluxed for 12 h and the particles were then purified by washing with acetonitrile. Purified intermediate particles (2.85 g) were dispersed in acetonitrile containing succinimid anhydride (10 g) and refluxed for 12 h followed by filtration and washing with acetonitrile. Particles were redispersed in acetonitrile (100 ml) and 1 g of Me5ASA was added. The mixture was refluxed for 12 h. Final particles were purified by filtration and washing steps with acetonitrile. Fluorescent SiNP were prepared accordingly while fluoresceinamine was used instead of Me5ASA.

Thin layer chromatography analysis (eluant: ethyl acetate/hexane 7:3 v/v) was used to confirm coupling of Me5ASA–SiNP. The structural analyses were performed via infrared spectroscopy (Shimadzu FTIR-8201 PC, Japan).

NP were analysed for diameter, size distribution, and zeta potential in 1:15 (v/v) dilutions with distilled water using a Zetasizer (Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK).

Atomic force microscopic imaging was conducted by use of a Nanoscope III with Bioscope IV controller (Digital Instruments, USA). All experiments were conducted at room temperature in air in Tapping Mode™ with NSC 16 tips at a resonant frequency of approximately 200 kHz. A droplet of the sample dissolved in deionized water (20 μl) was placed on a freshly cleaned cover slide and was allowed to dry before being subjected to AFM investigations.

2.2. In vitro drug release

50 mg of lyophilized drug-loaded NP were re-suspended in a 15 ml flask containing either phosphate buffer (pH 7.4) or artificial intestinal fluid (according to US Pharmacopoeia 23) and incubated at 37 °C under magnetic stirring (200 rpm). At appropriate intervals, 0.5 ml samples were withdrawn, centrifuged at 15,000 g for 30 min, and replaced by fresh buffer. Drug loads were determined by dispersing the same amount of NP in 1 M NaOH at 37 °C until particles were completely degraded. All experiments were performed in triplicate. The drug release was quantified by use of an isocratic high performance liquid chromatographic method developed to our specific requirements allowing simultaneous detection of both, 5ASA and Me5ASA. An SG5-ODD1-15QS 150 × 4.6 mm, 5 μm column (Interchim, France) constituted the stationary phase while the mobile phase consisted of acetonitrile–citric acid 0.01 M buffer pH 2 in a ratio 2:98 at a flow rate of 1 ml/min. Both, 5ASA and Me5ASA were detected by UV absorbance at 340 nm. Samples of 20 μl were injected into the column. The limit of detection of 5ASA and of Me5ASA was approximately 1 μg/ml. The retention time of 5ASA and Me5ASA was circa 3.5 and 7.4 min, respectively.

2.3. NP toxicity test in cultured cells

Caco-2 and HEK cells were seeded in 96-well plates (approximately 10,000 cells per well) and grown in Dulbecco's Modified Eagle Medium for one week. Thereafter, cells were incubated with the various preparations at different dilutions for 24 h. After carefully removing the supernatant and two washing steps with Krebs–Ringer buffer, cell viability was determined via MTT test. Cytotoxicity was expressed as a percentage of controls (untreated cells).

2.4. Colitis model

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 mouse model was chosen as a well-recognized experimental model [18] that allows induction of a colitis at an exact location. Male BALB/c mice (average weight 25 g, $n = 6$/group) were used for the inflammation model where inflammation was induced by TNBS via the following procedure: animals were catheterized 4 cm intrarectally after light narcotizing with ether. 100 μl of TNBS in ethanol were applied in a dose of 160 mg/kg body weight. The mice were housed for a day without treatment to establish the full model colitis.

During the treatment period, all animals received orally either 0.1 ml of 5ASA solution (at a dose of 30 or 100 mg/kg body weight) or Me5ASA–SiNP suspensions once daily for six consecutive days. The control groups received saline only (colitis control) or blank SiNP. The animals were sacrificed 24 h after the last drug/particle administration and their colons were resected.

2.5. Confocal laser scanning microscopy for the qualitative localization of SiNP

A Biorad MRC 1024 Laser Scanning Confocal Imaging System (Hemel Hempstead, UK), equipped with an argon ion laser (American Laser Corp., Salt Lake City, UT, USA) and a Zeiss Axiovert 100 microscope (Carl Zeiss, Oberkochen, Germany), was used to qualitatively detect the fluorescent particles in the tissue sections. The laser was adjusted in the green fluorescence mode, which yielded an excitation wavelength at 488 nm.

2.6. Pathophysiological parameters

The degree of inflammation was quantified by use of a clinical activity index assessing weight loss, stool consistency, and rectal bleeding as previously described elsewhere [19].

Resected colon tissue samples were opened longitudinally and rinsed with iced phosphate buffer to remove luminal content. Then tissue wet weight and colon length were determined and expressed as a colon weight/length quotient [20]. The measurement of myeloperoxidase activity was performed to quantify the severity of the colitis. Myeloperoxidase activity is a reliable index of severity of inflammation