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Lectin-decorated nanoparticles enhance binding to the inflamed tissue in experimental colitis☆

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ABSTRACT

A major limitation in the drug treatment of inflammatory bowel disease is the inability to deliver the drug selectively towards the inflamed tissues. Nanotechnology-based drug delivery systems have led to an amelioration of the therapeutic selectivity but still the majority of the entrapped drug is eliminated without exercising a therapeutic effect. Here, lectin-decorated drug loaded nanoparticles (NP) are suggested for active targeting and selective adhesion to the inflamed tissue in experimental colitis. Peanut (PNA) and wheat germ (WGA) lectins were covalently bound to the surface of NP and were tested for their stability and degree of bioadhesion in cell culture. In-vivo, the selectivity of bioadhesion and distribution of NP throughout the intestinal tract as well as the therapeutic benefit for glucocorticoid loaded lectin-NP was studied in murine colitis models. Quantitative adhesion analyses showed that lectin-conjugated NP exhibited a much higher binding and selectivity to inflamed tissue compared to plain NP (PNA conjugates: 52.2 ± 5.6%; WGA conjugates: 22.0 ± 0.8%; plain NP: 18.6 ± 9.8%). Lectin-associated NP revealed a further increase in the selectivity of bioadhesion towards inflamed tissues which partially translates into increased therapeutic efficiency. In terms of therapeutic efficiency, all glucocorticoid containing formulations revealed an enhanced therapeutic effect with lectin conjugates especially PNA-NP (myeloperoxidase: 55 ± 37 U/g; TNF-alpha: 3880 ± 380 U/g) compared to plain NP (myeloperoxidase: 145 ± 98 U/g; TNF-alpha: 6971 ± 1157 U/g). Targeted NP by using lectins, especially with PNA, as stable targeting moiety in the gastrointestinal tract appears to be a very promising tool in future treatment of inflammatory bowel disease.

1. Introduction

The general principle of drug therapy in IBD (inflammatory bowel disease) is to induce remission of acute attacks and to prevent acute attacks during remission. A large variety of anti-inflammatory drugs are commonly used to control acute attacks of the disease and to maintain remission [1]. Due to severe adverse effects drugs preferentially need to be made available at the inflammation site and to the healthy tissue which may risk systemic absorption. Unfortunately, most of the currently available therapeutic strategies lack this selectivity.

Although many efforts have been made for a higher specificity of drug by designing new drug delivery devices [2,3] all commercialized delivery systems fail in terms of selectivity [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. Among several new therapeutic approaches, nanoparticulate drug delivery systems have been proposed to mitigate this lack of selectivity [5–8]. This approach targets 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 inflammatory bowel disease were proposed. It was proven that nanoparticle uptake into those immune-related cells or the disrupted intestinal barrier at ulcerated regions allow the selective accumulation in the targeted area [9] subsequently followed by an enhanced therapeutic outcome [5,6,8,10,11].

This delivery concept based on passively targeting immune related cells in inflammation was successful in several different inflammatory diseases of the gastrointestinal tract and led to the postulation of the epithelial 'Enhanced Permeability and Retention' effect (EPR) [7,12]. Although the EPR concept was first reported in tumor targeting based on the increased permeability of the endothelium surrounding the tumor tissue based on the accompanying inflammatory reaction, this concept is not limited to tumor therapy but a universal phenomenon where permeability of the endothelial barrier is increased [13]. Therefore, also an

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anti-inflammatory therapy can be achieved by this approach after intravenous administration. Based on similar observations on the epithelium in the case of an inflammation an 'epithelial EPR effect' has been suggested [7,12].

However, also visible from these studies is the fact that 70 to 80% of administered drug is eliminated by the feces and does not contribute to the therapeutic efficiency at all [9]. A stronger interaction with the targeted tissue is therefore desirable and could significantly enhance the therapeutic outcome. Active targeting provided by a specific recognition moiety on the particle surface may increase the adhesion and the specificity at the same time. Such active targeting has been suggested after intravenous administration in many pathologies and usually relies on an association of antibodies with the particle surface which enables the specific interaction with a certain target cell [14–16].

Unfortunately, antibodies are unstable in the intestinal fluid and undergo proteolytic or pH-dependent denaturation and therefore such an approach is hardly applicable in the gastrointestinal tract. An alternative could be lectins, glycoproteins capable of specific recognition and of reversible binding to carbohydrate moieties of complex glycoconjugates. They are often resistant to degradation by heating and digestive processes [17].

We report here the design of lectin-decorated drug loaded NPs highlighting the specificity of tissue binding followed by an evaluation of the therapeutic benefit of this new actively targeted drug delivery system for the treatment of IBD.

Wheat germ agglutinin (WGA) and peanut agglutinin (PNA) were selected as two candidates for an active targeting moiety due to sufficient binding to the gastrointestinal mucosa [18–21] and their relatively good resistance to acidic pH and enzymatic degradation, but also of their low cytotoxicity [22,23]. While WGA is providing a rather non-specific binding to the mucosa throughout the entire gastrointestinal tract, PNA is a good candidate for specific targeting to the inflamed tissue [17].

2. Materials and methods

2.1. Materials

Micro BCA Protein Assay Kit (μ-BCA), Cryomatrix™ gel and betamethasone drug (BMS) were purchased from Thermo Scientific (Rockford, USA) and Sigma-Aldrich (France) respectively. Polystyrene fluorescent nanoparticles (PS-NPs) were purchased from Polysciences Inc. (Eppenheim, Germany). Ethyl acetate and 2-mercaptoethanol was provided by Carlo Erba Reagents (France) and VWR International (PA, USA), respectively. Resomer RG 502H® (PLGA, lactide: glycolide = 50:50) was obtained from Boehringer Ingelheim (Germany). Peanut Agglutinin (PNA) and Wheat Germ Agglutinin (WGA) were the products of Vector Laboratories (CA, USA). Sensolyte® pNPP Alkaline Phosphatase Assay Kit and a RayBio® Mouse TNF-alpha Elisa Kit were received from MoBiTec (Goettingen, Germany) and RayBiotech, Inc. (Norcross GA, USA) respectively.

All other chemicals were obtained from Sigma Aldrich (Deisenhofen, Germany) or Gibco Life Technologies (Grand Island, NY, USA) and were of analytical grade. Caco-2 and RAW 264.7 macrophage-like cells (ATCC TIB-71) were purchased from the American Type Culture Collection (ATCC) (Rockville, MD, USA).

2.2. Preparation of betamethasone-loaded PLGA-NPs

NPs were prepared by an oil-in-water solvent evaporation method (simple oil/water emulsification technique). Briefly, 10 mg of betamethasone (BMS) and 100 mg of PLGA 50:50 were dissolved in 5 g of ethyl acetate under magnetic stirring. The whole organic phase was then emulsified by sonication in 15 ml of 0.1% polyvinyl alcohol (PVA) aqueous solution in an ice bath. Ethyl acetate was evaporated overnight at room temperature under magnetic stirring. Drug-free PLGA-NPs were prepared in a similar way.

2.3. Preparation of lectin-conjugated PLGA-NPs or lectin-conjugated polystyrene fluorescent nanoparticles (PS-NPs)

For the lectin-NP conjugates adhesion assessment in-vitro or binding studies on intestinal tissue in-vivo and, PS-NPs (nominal size of 500 nm) from Polysciences Inc. (Germany) were used. Lectins were covalently coupled to NPs using the carbodiimide (EDC) method with slight modification [24].

Briefly, 1.5 ml-aliquots of the NP suspension were washed once with 100 mM MES buffer, pH 5.2 by centrifugation at 15,000 *g* during 5 min. NPs were then incubated with approximately 50 mM EDC and 150 mM sulfo-NHS in MES buffer, pH 5.2. The reaction was performed 1 h at room temperature. To quench the EDC excess, 20 mM of 2-mercaptoethanol was added and supernatant was discarded after centrifugation. Then, the pellet was resuspended in Dulbecco's Phosphate Buffered Saline (DPBS), and PNA or WGA in DPBS at a concentration of 1 mg/ml were incubated with NPs overnight under gentle agitation. To saturate the unreacted sites, 50 mM of glycine was incubated with NPs for 1 h. Finally, surface-modified NPs were washed twice with DPBS. As a control, lectins were replaced by bovine serum albumin (BSA) and conjugation was performed according to the same procedure.

2.4. Characterization of lectin-conjugated PLGA-NPs

The amount of unbound lectin was quantified after particle separation by colorimetric determination of protein in the supernatant by a μ-bicinchoninic protein assay (μ-BCA kit, Thermo Scientific, USA). Particle size was measured by light scattering using a Zetasizer Nano ZS (Malvern Instruments, UK). The betamethasone content was measured in freeze-dried NPs after the conjugation procedure. The freeze-dried samples were dissolved in methylene chloride and vortexed for 5 min to extract betamethasone. Samples were centrifuged at 15,000 *g* for 2 min and betamethasone was quantified at 238 nm in the supernatant using an UV/Vis spectrophotometer Specord 205 (Shimadzu, France).

2.5. Stability of PNA and WGA conjugates in simulated gastric and intestinal fluids

Simulated gastric fluid with or without pepsin (SGF) and simulated intestinal fluid with or without pancreatin (SIF) were prepared according to the US Pharmacopeia 36 [25]. The lectin conjugates were incubated at 37 °C in these different media for 2 h in order to analyse the stability of the targeting moiety in gastrointestinal tract. Samples were centrifuged twice for 5 min at 15,000 *g*, supernatant was discarded and the pellet was washed with HBSS buffer. Thereafter, the particle pellet was resuspended in HBSS buffer at a final concentration of 0.26 mg/ml and tested on caco-2 and RAW 264.7 macrophages as described earlier.

2.6. Cell culture

To examine the in vitro cell binding of lectin conjugates, assays were performed at 4 °C and 37 °C. At 4 °C active transport processes are reduced to a minimum. Thus the cell-associated fluorescence intensity acquired predominantly refers to surface binding of PS-NPs. At 37 °C, active transport may occur due to active processes and increased membrane fluidity, thereby promoting cellular uptake [26].

Cells were cultured using DMEM + Glutamax medium supplemented with 10% FCS and 1% penicillin–streptomycin solution for macrophage cells, and with 15% FCS, 1% MEM NEAA and 1% penicillin–streptomycin solution for caco-2 cells, the 50 ml cell culture flask was used. Cells were cultivated in an incubator at 37 °C with 5% carbon dioxide.

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