EPITHELIAL ACTIVE TARGETING BY LECTIN-NANOPARTICLES IN MURINE EXPERIMENTAL COLITIS

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ABSTRACT
The treatment of inflammatory bowel disease (IBD) is limited due to the inability of the drug to target selectively the diseased tissues. In this study, lectins conjugated to betamethasone-loaded nanoparticles have been developed for the active targeting of inflamed tissue. Results show a significant improvement of the therapeutic activity of the betamethasone entrapped within lectin-grafted nanoparticles compared with untargeted nanocarriers.

INTRODUCTION
One of major requirements for the colitis treatment is to selectively deliver drugs to inflamed tissue. The aim is to improve the therapeutic potential of the drug and to reduce the adverse effects. Although many efforts have been made, all marketed delivery systems did not demonstrate a high level of selectivity. However, earlier studies showed an increased adhesion of small particulate drug carriers to the inflamed tissue of the gastrointestinal tract. This result supports the concept of a particle size-mediated targeting in ulcerative colitis. The present study concerns the development of a nanoparticulate drug delivery system for the active targeting of inflamed tissues. In IBD, adhesive properties were observed for certain lectins, namely peanut agglutinin (PNA) which was selected to be coupled to NP due to their ability to recognize specific molecules of the inflamed mucosal tissue.

EXPERIMENTAL METHODS

In vitro design particles
Nanoparticles (NPs) were prepared by adjusting a simple oil/water emulsification technique. Briefly, 100mg of Poly (D,L-lactic-co-glycolic) (PLGA, 50:50) and 10 mg of betamethasone (BMS) were simultaneously dissolved in 5 g of ethyl acetate. The organic solution was then added into 15 ml of poly (vinyl alcohol) (PVA) 0.1% aqueous solution. The preparation was emulsiﬁed by sonication during 3 minutes in an ice bath. Finally, the solvent was evaporated overnight under gentle stirring.

Lectin-NP coupling
Lectin [Peanut agglutinin (PNA) or Wheat germ agglutinin (WGA)] was covalently coupled to the surface of nanoparticles by the carbodiimide method. Firstly, Carboxylic of PLGA NP were activated with 1 of 1-[3-(Dimethylamino)-propyl]-3-ethyl carbodiimide hydrochloride (EDC) and 3.4% of (N-hydroxysulfosuccinimide) (sulfo-NHS) in MES buffer at pH 5.2 during 1 h at room temperature. EDC were then inactivated by addition of 20 mM of beta-mercaptoethanol. After removing the supernatant by centrifugation, the activated NPs were incubated with lectins overnight at room temperature under gentle stirring. Unreacted sites of PLGA NP were blocked by addition of 50 mM of glycine. Final conjugates were washed with saline phosphate buffer and stored at 4°C.

Colitis model
A colitis model was induced to mice by trinitrobenzo-sulfonic acid (TNBS). 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. Clinical activity score, colon weight/length index, myeloperoxidase (MPO) and alkaline phosphatase (AP) activity were determined to assess the inflammation after different treatments with betamethasone (BMS).

RESULTS AND DISCUSSION

Figure 1: Accumulation behavior of lectin-NP or NP into healthy or inflamed tissue.
The quantitative adhesion analyses showed that lectin-NP exhibited three-fold higher adhesion to inflamed tissue compared to plain NP (Figure 1). The best adhesion results were obtained with PNA lectin whatever the administration route (oral or rectal) (Figure 1).

In terms of therapeutic efficiency, all Betamethasone (BMS) containing formulations showed a myeloperoxidase and alkaline phosphatase activity different from the untreated group. Enzyme activities revealed the higher therapeutic effects with PNA-NP-BMS or WGA-NP-BMS than NP-BMS (Figures 2 and 3). PNA-NP demonstrated higher anti-inflammatory activity than WGA-NP. However after rectal administration, (circumventing the intestinal passage), conjugates or plain NPs were locally applied which explains that the results obtained showed an enormous therapeutic efficiency which was superior to the oral route. In all cases NPs without BMS were without therapeutic effect and results obtained were comparable to untreated colitis.

The conjugates exhibited a higher adhesion to inflamed tissue and also an increasing therapeutic efficiency of the associated amount of drug. Lectin-NPs demonstrate a further increase in efficiency and selectivity and suggest active nanoparticle targeting as an promising tool in future treatment of IBD.

REFERENCES

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Figure 2: Myeloperoxidase (MPO) activity on day 8 in TNBS colitis model after oral or rectal administration of NP-BMS and lectin-NP-BMS.

Figure 3: Alkaline phosphatase (AP) activity on day 8 in TNBS colitis model after oral or rectal administration of NP-BMS and Lectin-NP-BMS.