Targeted Delivery of NK007 to Macrophages to Treat Colitis

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Received 10 February 2015; revised 26 March 2015; accepted 15 April 2015

Published online 11 May 2015 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24473

ABSTRACT: Macrophages are important therapeutic targets for various disorders, including infectious diseases, inflammatory diseases, metabolic diseases, and cancer. In this study, we report a novel oral delivery system for the targeted delivery of anti-inflammatory therapeutics to macrophages. Using this formulation, the model drug tylophorine malate (NK007) was tightly incorporated inside beta-glucan particle shells by the formation of colloidal particles with chitosan, tripolyphosphate, and alginate via electrostatic interactions. This formulation specifically delivered NK007 to macrophages *in vivo* after oral gavage and effectively cured colitis in the dextran sulfate sodium-induced murine colitis model, highlighting the utility of beta-glucan particles as an oral anti-inflammation drug delivery system by targeting macrophages. In this work, NK007 was selected as the model drug. However, this novel oral carrier system has the potential to be applied as a platform for the treatment of many other diseases for which macrophages are the therapeutic targets. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2276–2284, 2015

Keywords: macrophage; targeted drug delivery; colonic drug delivery; oral absorption; encapsulation; biodegradable polymers; colitis; inflammatory cytokine; glucan particle

INTRODUCTION

Macrophages play important roles in the pathophysiology of numerous disorders.[1–3] In particular, macrophages form an important line of defense against bacterial and viral infection,[4,5] but have detrimental functions in chronic inflammatory diseases such as bowel disease,[6] rheumatoid arthritis (RA),[7] and multiple sclerosis[8] as well as in metabolism diseases, atherosclerosis,[9] and cancer.[10,11] Thus, macrophages are well-known therapeutic targets for various diseases.[12–14] For example, the inflammatory cytokine tumor necrosis factor-alpha (TNF-α) is produced by activated macrophages or dendritic cells and plays a central role in acute and chronic inflammatory diseases such as RA,[15] inflammatory bowel disease (IBD),[16] and bacterial septic shock.[17] The inhibition of this cytokine with anti-TNF-α antibodies (infliximab and adalimumab)[18,19] or a recombinant-soluble TNF receptor–Fc fusion protein (etanercept)[20] has been most widely applied to the treatment of patients with RA or IBD. Recently, tylophorine malate (NK007), a small-molecule compound, was found to have an extraordinary inhibitory activity against TNF-α production, thus endowing it with a great potential for the treatment of IBD[21] and RA,[22] particularly considering the high cost and inconvenient administration routes (intravenous or intramuscular) of biological agents.

For the treatment of diseases involving macrophages, particularly chronic diseases, mechanisms for reducing the serious side effects of chronic medication have practical importance for clinical applications. First, researchers try to improve drug selectivity to reduce off-target effects through chemical modifications. Second, the targeted delivery of drug molecules to macrophages is a practical alternative; this method could potentially enable new treatment paradigms for numerous diseases.[23] Once the drug molecules are encapsulated into the carrier systems, the amount of therapeutic agent needed to obtain a clinical effect may be reduced, thereby potentially reducing drug-induced toxicity and other side effects.[24] Extensive studies have attempted to fabricate carrier systems for macrophage-targeting purposes.[25–27] In general, however, practical clinical considerations such as scale-up capability, cost, storage, biocompatibility, and target specificity make the development of specific delivery systems to macrophages challenging. First, very complex processes are required to express pattern recognition receptor ligands, such as peptides, antibodies, lectins, and polysaccharides, on the carrier surface, and the laborious and complex procedure is not appropriate for scale-up.[28–30] Second, the fabricated particles are extremely heterogeneous in size and surface constitution, which creates nonconsistent biological results when used in clinics.[31] Third, the fabricated particles aggregate or degenerate during storage and exhibit low biocompatibility for clinical use. These shortcomings greatly restrict their clinical potential.

Most targeted delivery systems can only be utilized through intravenous administration. Compared with intravenous administration, oral drug delivery is more favorable and can provide long-term, continuous drug exposure at disease sites at relatively lower and safer concentrations,[32] which is particularly more appropriate for chronic treatment regimens.[33,34] However, oral drug delivery is a challenge, as evidenced by the number of published studies on the intravenous administration of particle systems compared with the few studies on oral administration.[35,36] For these reasons, cheaper and more applicable systems, particularly oral delivery systems, are required for targeting macrophages.

Beta-glucans are generally recognized as safe, and are widely used as supplements in human nutrition or food.[37] They are very cheap and can be supplied in large quantities. As the major yeast pathogen-associated molecular pattern, beta-glucans

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Journal of Pharmaceutical Sciences, Vol. 104, 2276–2284 (2015)
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are recognized by the pattern recognition receptor Dectin-1, a highly expressed lectin on the surface of phagocytic macrophages.[38] Therefore, beta-glucans can be efficiently and specifically taken up by macrophages.[39–41] Beta-glucans prepared from the yeast cell wall exist as two classes of particles: glucan particles (GPs), which are primarily composed of beta-1,3-D-glucan, and glucan mannan particles (GMPs), which contain both beta-1,3-D-glucan and mannoproteins in the outer cell wall. The 2–4 μm uniform microsphere particle size favors their uptake by M cells in Peyer's patches and subsequent dissemination to the mesenteric lymph nodes, blood circulation, and spleen, which may permit the delivery of these molecules through oral gavage.[42] Furthermore, their hollow and porous structure permits high drug loading. Therefore, beta-glucan particles, including both GPs and GMPs, have the potential to be an ideal platform for macrophage-specific drug delivery. For example, GMPs were successfully utilized by Aouadi and coworkers[42,43] to encapsulate small interfering RNA to form an efficient oral delivery vehicle for targeting macrophages, and the formed particles potentially protected animals from lipopolysaccharide (LPS)-induced death. Huang et al.[44], used GPs to form a protein vaccine carrier via the internal absorption of a protein antigen that was used for immunologic stimulation *in vivo*.[45] We successfully used exceptionally biocompatible materials, including chitosan and alginate, to cage proteins inside GMPs to form a practical protein delivery system to macrophages.[46]

Here, we attempted to use GMPs to fabricate a potential oral carrier system for the specific delivery of the small compound NK007 to macrophages for the treatment of inflammatory diseases. In this formulation, chitosan, tripolyphosphate (TPP), and alginate were used to form colloidal particles with the model drug NK007 via electrostatic interactions that incorporated NK007 tightly within GMPs. The NK007-encapsulated GMPs (GMP-NK007) delivered NK007 to macrophages *in vitro* and *in vivo*. A dextran sulfate sodium (DSS)-induced murine colitis model that resembles human ulcerative colitis was utilized to evaluate the effects of the formed particles delivered via oral gavage, and the results highlighted the utility of this particular system as an efficient oral delivery system targeted to macrophages.

MATERIALS AND METHODS

Materials

Low guluronic content, low-viscosity sodium alginate (100–200 mPa s), and low-molecular-weight (MW) chitosan (MW 10–20 kDa) were purchased from Heowns (Tianjin, P. R. China). NK007 was synthesized according to the procedure described in our previous study.[47] Prednisone was purchased from Lisheng Chemical Company (Tianjin, P. R. China). LPS (*Escherichia coli* serotype 0111:B4) was obtained from Sigma (St. Louis, Missouri). DSS (MW 36–50 kDa) was purchased from MP Biomedical (Solon, Ohio). Fecal occult blood test kit was purchased from Baso (Zhuhai, P. R. China). Mouse TNF-α enzyme-linked immunosorbent assay (ELISA) kit was purchased from Biolegend (San Diego, California). Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Invitrogen Life Technologies (Carlsbad, California). All other chemicals and reagents used were of analytical grade and obtained commercially unless stated otherwise.

Cells and Animals

The murine macrophage cell line RAW 264.7 was purchased from Saierbio (Tianjin, P. R. China) and cultured in RPMI-1640 medium. All cells were incubated in medium containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in humidified air supplemented with 5% CO_2. Wild-type BALB/c mice (8–10 weeks of age) and wild-type C57BL/6J mice (8–10 weeks of age) were purchased from Academy of Military Science (Beijing, P. R. China). All mice were fed with standard food and received humane care in accordance with the animal care provision in the animal facility of College of Life Sciences, Nankai University (Tianjin, P. R. China).

Preparation of GMPs

Glucan mannan particles were prepared from commercially available *Saccharomyces cerevisiae* baker's yeast (Angel Yeast Company, Ltd., Inner Mongolia, P. R. China) via a series of alkaline and acidic extraction steps as described previously.[39,46] Briefly, 3.75 g baker's yeast was resuspended in 50 mL water, and the pH was adjusted to 12.0–12.5 with 1.0 M NaOH. The suspension was heated to 60°C with stirring for 1 h and then centrifuged to recover the insoluble material containing the cell walls. The material was then resuspended in 50 mL water, brought to pH 4–5 with HCl, and heated to 55°C with stirring for an additional 1 h. GMPs were collected after centrifugation, successive washes with water (three times), isopropanol (four times), and acetone (two times) and drying under a vacuum to yield 1.72 g slightly off-white powder.

Rhodamine B Labeling of GMPs

Glucan mannan particles were labeled with rhodamine B isothiocyanate (Rho. B). Briefly, GMPs (1.0 g) were incubated with Rho. B (10 mg, dissolved at 2.5 mg/mL in dimethyl sulfoxide (DMSO)) in 100 mL sodium carbonate buffer (0.1 M, pH 9.2) overnight at 37°C in the dark. Unreacted Rho. B was then quenched by incubation with Tris buffer (10 mL, 1.0 M, pH 8.3) for 30 min. The labeled GMPs were extensively washed with sterile water until the color was removed, dehydrated with absolute ethanol and acetone, and then dried under vacuum in the dark at room temperature. The Rho. B-labeled GMPs were used for confocal imaging in *in vitro* cellular uptake assay and *in vivo* oral gavage assay.

Preparation of GMP-NK007

NK007-encapsulated GMP particles were prepared based on the ionic gelation of chitosan with TPP and alginate sodium inside the GMPs. Briefly, 100 mg dry GMPs were mixed with 500 μL NK007 solution (20 mg/mL in 5 mg/mL low-molecular-weight chitosan solution) at room temperature for 2 h to allow the particles to swell and engulf the NK007 and chitosan solution. The samples were then frozen at −80°C and lyophilized. To maximize NK007 incorporation into the GMP shells, the dry GMPs were swollen by mixing with 500 μL of sterile water for 2 h and relyophilized. TPP–ALG solution (500 μL, 1.0 mg/mL TPP and 0.4 mg/mL sodium alginate) was added for incubation of 30 min and then 5 mL TPP–ALG solution was added for 1 h to complete the complexation, through which NK007 was trapped inside the GMP shells. The formed particles were dispersed by ultra-sonication for 5 min and stirring for 1 h, centrifuged at 2000*g* for 5 min, washed with phosphate-buffered

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