# Drug-Loaded Nanoparticles Targeted to the Colon With Polysaccharide Hydrogel Reduce Colitis in a Mouse Model

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**BACKGROUND & AIMS:** One of the challenges to treating inflammatory bowel disease (IBD) is to target the site of inflammation. We engineered nanoparticles (NPs) to deliver an anti-inflammatory tripeptide Lys-Pro-Val (KPV) to the colon and assessed its therapeutic efficacy in a mouse model of colitis. **METHODS:** NPs were synthesized by double-emulsion/solvent evaporation. KPV was loaded into the NPs during the first emulsion of the synthesis process. To target KPV to the colon, loaded NPs (NP-KPV) were encapsulated into a polysaccharide gel containing 2 polymers: alginate and chitosan. The effect of KPV-loaded NPs on inflammatory parameters was determined in vitro as well as in the dextran sodium sulfate–induced colitis mouse model. **RESULTS:** NPs (400 nm) did not affect cell viability or barrier functions. A swelling degree study showed that alginate-chitosan hydrogel containing dextran–fluorescein isothiocyanate–labeled NPs collapsed in the colon. Once delivered, NPs quickly released KPV on or within the closed area of colonocytes. The inflammatory responses to lipopolysaccharide were reduced in Caco2–BBE (brush border enterocyte) cells exposed to NP-KPV compared with those exposed to NPs alone, in a dose-dependent fashion. Mice given dextran sodium sulfate (DSS) followed by NP-KPV were protected against inflammatory and histologic parameters, compared with mice given only DSS. **CONCLUSIONS: Nanoparticles are a versatile drug delivery system that can overcome physiologic barriers and target anti-inflammatory agents such as the peptide KPV to inflamed areas. By using NPs, KPV can be delivered at a concentration that is 12,000-fold lower than that of KPV in free solution, but with similar therapeutic efficacy. Administration of encapsulated drug-loaded NPs is a novel therapeutic approach for IBD.**

*Keywords:* Colon Targeting; Colitis; KPV-Loaded Nanoparticle; Polysaccharide Hydrogel.

Inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis, is a relapsing and remitting chronic disease for which treatment options are limited. Most existing treatments are associated with significant side effects.[1–4] Newer targeted treatments such as anti–tumor necrosis factor (TNF) agents are effective in a subset of patients but have to be administered systemically and also are associated with significant side effects.[1] A major advance in therapeutic strategies in diseases such as IBD would be the ability to target drugs to the site of inflammation in sufficient quantities to maximize local drug concentration and minimize systemic side effects. However, targeting drugs to the site of inflammation has remained a challenge in IBD because of the lack of vehicles that could carry sufficient drugs or that could be released at the site of inflammation. Another problem is created by organs of the gastrointestinal tract, particularly the colon, because it is a challenge to deliver the drug to the colon with minimal digestive enzyme degradation and/or systemic absorption.[5] Various carriers have been designed to release the drug at a specific pH value, to be resistant to digestive enzymes, and/or require bacterial cleavage for activation, and several of these carriers currently are being investigated.[6–8] However, most of these drugs need to be administered in large doses, multiple times a day, resulting in poor patient compliance.

Among the various carriers proposed for drug delivery, polymeric nanoparticles (NPs) have been studied for several decades. The most widely used polymers to engineer NPs are aliphatic polyesters such as polylactide (PLA),[9] polyglycolide, and co-polymers,[10,11] which have been approved by the US Food and Drug Administration and have well-characterized biocompatibility and (bio)degradability properties.[12,13]

Recent studies have reported using nanotechnology for cell targeting. Thus, micelles have been described as promoting the uptake of 40-nm colloidal gold particles,

*Abbreviations used in this paper:* BSA, bovine serum albumin; COX-2, cyclooxygenase-2; DSS, dextran sodium sulfate; ECIS, electrical impedance sensing; FITC, fluorescein isothiocyanate; IL, interleukin; LPS, lipopolysaccharide; MPO, myeloperoxidase; NP, nanoparticle; PCS, photon correlation spectroscopy; PLA, polylactide; PVA, polyvinyl alcohol; TNF, tumor necrosis factor.


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particularly in the rectal area.[14] Destruction of extracellular matrix (colonic mucosal) in the apical side of epithelia and alterations in the barrier function organization, mainly caused by destruction of epithelia tight junctions, dramatically increase the potential permeability and interactions of the epithelia to NPs. As described,[15] patients with ulcerative colitis showed a 15-fold greater uptake of Evans Blue dye in the plasma of treated individuals vs control individuals. In addition, after administration by enema, colonic absorption of both uncoated and ligand-modified latex NPs has been reported. This uptake seems to be size-dependent; approximately 16% of 100-nm polystyrene particles were absorbed over a 10-day period, whereas only approximately 2% of 1-$\mu$m microparticles were absorbed under the same conditions.[16] The attachment of bioadhesive tomato lectin to 500-mm NPs decreased uptake by rat colonic epithelial cells by 7-fold, indicating an alteration in surface particle characteristics or a lack of appropriate receptors on the colonic epithelia.

In the present study, we sought to deliver drug-loaded NPs to the colon. We used the tripeptide KPV as an anti-inflammatory drug because it has been shown that KPV, added to drinking water at 205 $\mu$g/day (assuming a water uptake of 6 mL[17] of a 100-$\mu$mol/L KPV solution) or administrated intraperitoneally at 10 $\mu$g/day, decreased colitis in mice.[18–20] Furthermore, we compared the effective KPV dose when delivered in free solution with the effective dose when loaded onto NPs in the induced-colitis model. With the aim of packaging the KPV-loaded NPs into a biocompatible and biodegradable polymer, we sought to identify a biomaterial made of polysaccharides that degrades in the colon and thus delivers the KPV-loaded NPs to the inflamed colonic area. The biological properties of polysaccharides have been used for decades in applications such as wound healing, cell encapsulation, or as an oral delivery vehicle.[21–24] We used a polysaccharide gel containing 2 polymers, alginate and chitosan, as a vector. Chitosan is a biocompatible and biodegradable polymer.[25,26] Alginate and chitosan form gels that chelate with a calcium or sulfate solution, respectively.[27] The combination of alginate and chitosan chelation should maintain the 3-dimensional form of the bead and the chitosan also has therapeutic effects on inflammatory cells in the colon.[27,28]

## Materials and Methods

### *Preparation of Nanoparticles Loaded With KPV and Characterization*

See Supplementary Materials and Methods section.

### *Size Determination of the KPV-Loaded NPs*

See Supplementary Materials and Methods section.

### *Scattering Electron Microscopy of NPs*

See Supplementary Materials and Methods section.

### *NP-Loaded KPV*

KPV and bovine serum albumin (BSA) form a homogenous internal aqueous phase (see the NP synthesis process described in Figure 1). To measure KPV loaded onto NPs, BSA concentration was measured by ultraviolet spectroscopy in the final washing solutions. The encapsulation rate of BSA then was determined based on the initial BSA concentration. After each final washing to remove extra polyvinyl alcohol (PVA) by centrifugation of NP suspension, the supernatant was collected. The accumulated washing volumes were used to determine the concentration of BSA present in the supernatant, thus providing the mass of protein not encapsulated. A mass balance was performed to determine the amount of BSA that was loaded into the NP according to the known initial concentration.

The amount of BSA is determined by ultraviolet quantification at 280 nm: $A = \log (I_0/I) = \varepsilon.l.c$, where A is the solution absorbance, Io is the initial intensity, I is the intensity after sample, $\varepsilon$ is the absorbance coefficient of BSA (L/g.cm), l is the cuvette length (cm), and c is the BSA concentration (g/L).

Finally, the encapsulation rate was used to determine the KPV loading, assuming that KPV and BSA are loaded in the same way in a homogenous aqueous phase: encapsulation rate = 71% ± 4%. This rate means that the loaded KPV concentration (in ng) compared with the dry weight in NPs (in mg) is 84 ± 3.3 ng/mg.

To compare KPV-loaded NPs with free KPV, we calculated a KPV concentration in water that was equivalent to a 500-$\mu$g/mL concentration of KPV-loaded NPs. The calculated free KPV solution was equivalent to an 814-fold dilution of a 100-$\mu$mol/L initial KPV solution.

### *Encapsulation of KPV-Loaded NPs Into Biomaterials*

Chitosan powder was solubilized in acetic acid then neutralized by addition of NaOH (0.1 mol/L) to give a final chitosan concentration of 0.6% (wt/vol). Medium-viscosity sodium alginate was prepared in NaCl (0.15 mol/L) 1.4% (wt/vol). Before mixing with NaCl, or acetic acid, the polymer powders were weighed, placed in glass tubes, and autoclaved. Alginate solution and chitosan solutions were mixed at a 1:1 ratio for a final concentration of 7 and 3 g/L, respectively. The polymer suspension was homogenized for 24 hours.

Different NPs were added to obtain a 2-mg/mL concentration of hydrogel solution and stirred to disperse NPs throughout the polymer solution. A chelation solution containing 70 mmol/L of calcium chloride and 30 mmol/L of sodium sulfate was prepared. Figures 2 and 3 show the procedure for inclusion of loaded NPs into