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NANOPARTICLES FOR PROTEIN DRUG DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of U.S. patent application Ser. No. 11/029,082, filed Jan. 4, 2005, entitled "Nan particles for Paracellular Drug Delivery", now U.S. Pat. No. 7,265,090, which is a continuation in part application of U.S. patent application Ser. No. 10/958,864, filed Oct. 5, 2004, pending, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention is related to medical uses of nanoparticles composed of chitosan/poly- γ -glutamic acid with protein drugs and their permeability for enhanced paracellular drug delivery.

BACKGROUND OF THE INVENTION

Production of pharmaceutically active peptides and proteins in large quantities has become feasible (Biomacromolecules 2004;5:1917-1925). The oral route is considered the most convenient way of drug administrations for patients. Nevertheless, the intestinal epithelium is a major barrier to the absorption of hydrophilic drugs such as peptides and proteins (J. Control. Release 1996;39:131-138). This is because hydrophilic drugs cannot easily diffuse across the cells through the lipid-bilayer cell membranes. Attentions have been given to improving paracellular transport of hydrophilic drugs (J. Control. Release 1998;51:35-46). The transport of hydrophilic molecules via the paracellular pathway is, however, severely restricted by the presence of tight junctions that are located at the luminal aspect of adjacent epithelial cells (Annu. Rev. Nutr. 1995;15:35-55). These tight junctions form a barrier that limits the paracellular diffusion of hydrophilic molecules. The structure and function of tight junctions is described, inter alia, in Ann. Rev. Physiol. 1998;60:121-160 and in Ballard TS et al., Annu. Rev. Nutr. 1995;15:35-55. Tight junctions do not form a rigid barrier but play an important role in the diffusion through the intestinal epithelium from lumen to bloodstream and vice versa.

Movement of solutes between cells, through the tight junctions that bind cells together into a layer as with the epithelial cells of the gastrointestinal tract, is termed paracellular transport. Paracellular transport is passive. Paracellular transport depends on electrochemical gradients generated by transcellular transport and on solvent drag through tight junctions. Tight junctions form an intercellular barrier that separates the apical and basolateral fluid compartments of a cell layer. Movement of a solute through a tight junction from apical to basolateral compartments depends on the "tightness" of the tight junction for that solute.

Polymeric nanoparticles have been widely investigated as carriers for drug delivery (Biomaterials 2002;23:3193-3201). Much attention has been given to the nanoparticles made of synthetic biodegradable polymers such as poly- ϵ -caprolactone and polylactide due to their good biocompatibility (J. Drug Delivery 2000;7:215-232; Eur. J. Pharm. Biopharm. 1995;41:19-25). However, these nanoparticles are not ideal carriers for hydrophilic drugs because of their hydrophobic property. Some aspects of the invention relate to a novel nanoparticle system, composed of hydrophilic

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chitosan and poly- γ -glutamic acid hydrogels that is prepared by a simple ionic-gelation method. This technique is promising as the nanoparticles are prepared under mild conditions without using harmful solvents. It is known that organic solvents may cause degradation of peptide or protein drugs that are unstable and sensitive to their environments (J. Control. Release 2001;73:279-291).

Following the oral drug delivery route, protein drugs are readily degraded by the low pH of gastric medium in the stomach. The absorption of protein drugs following oral administration is challenging due to their high molecular weight, hydrophilicity, and susceptibility to enzymatic inactivation. Protein drugs at the intestinal epithelium could not partition into the hydrophobic membrane and thus can only traverse the epithelial barrier via the paracellular pathway. However, the tight junction forms a barrier that limits the paracellular diffusion of hydrophilic molecules.

Chitosan (CS), a cationic polysaccharide, is generally derived from chitin by alkaline deacetylation (J. Control. Release 2004;96:285-300). It was reported from literature that CS is non-toxic and soft-tissue compatible (Biomacromolecules 2004;5:1917-1925; Biomacromolecules 2004;5:828-833). Additionally, it is known that CS has a special feature of adhering to the mucosal surface and transiently opening the tight junctions between epithelial cells (Pharm. Res. 1994;11:1358-1361). Most commercially available CSs have a quite large molecular weight (MW) and need to be dissolved in an acetic acid solution at a pH value of approximately 4.0 or lower that is sometimes impractical. However, there are potential applications of CS in which a low MW would be essential. Given a low MW, the polycationic characteristic of CS can be used together with a good solubility at a pH value close to physiological ranges (Eur. J. Pharm. Biopharm. 2004;57:101-105). Loading of peptide or protein drugs at physiological pH ranges would preserve their bioactivity. On this basis, a low-MW CS, obtained by depolymerizing a commercially available CS using cellulase, is disclosed herein to prepare nanoparticles of the present invention.

The γ -PGA, an anionic peptide, is a natural compound produced as capsular substance or as slime by members of the genus *Bacillus* (Crit. Rev. Biotechnol. 2001;21:219-232). γ -PGA is unique in that it is composed of naturally occurring L-glutamic acid linked together through amide bonds. It was reported from literature that this naturally occurring γ -PGA is a water-soluble, biodegradable, and non-toxic polymer. A related, but structurally different polymer, [poly(α -glutamic acid), α -PGA] has been used for drug delivery (Adv. Drug Deliver. Rev. 2002;54:695-713; Cancer Res. 1998;58:2404-2409). α -PGA is usually synthesized from poly(γ -benzyl-L-glutamate) by removing the benzyl protecting group with the use of hydrogen bromide. Hashida et al. used α -PGA as a polymeric backbone and galactose moiety as a ligand to target hepatocytes (J. Control. Release 1999;62:253-262). Their in vivo results indicated that the galactosylated α -PGA had a remarkable targeting ability to hepatocytes and degradation of α -PGA was observed in the liver.

Thanou et al. reported chitosan and its derivatives as intestinal absorption enhancers (Adv Drug Deliv Rev 2001; 50:S91-S101). Chitosan, when protonated at an acidic pH, is able to increase the paracellular permeability of peptide drugs across mucosal epithelia. Co-administration of chitosan or trimethyl chitosan chloride with peptide drugs were found to substantially increase the bioavailability of the peptide in animals compared with administrations without the chitosan component.