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Polysaccharide based colon-specific nano particulate drug delivery system

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ABSTRACT

Natural polysaccharides are now extensively used for the development of solid dosage forms for delivery of drug to the colon. The rationale for the development of a polysaccharide based Nano particulate delivery system for colon. Various approaches used for targeting the drugs to the colon include, formation of a prodrug, multi-coating time-dependent delivery systems, coating with pH-sensitive polymers, pressure dependent systems, and the use of biodegradable polymers. In particular, polysaccharides seem to be the most promising materials in the preparation of nanometeric carriers. In this review, four mechanisms are introduced to prepare polysaccharides-based nanoparticles, that is, covalent crosslinking, ionic crosslinking, polyelectrolyte complex, and the self-assembly of hydrophobically modified polysaccharides. The approaches utilizing polysaccharides for colon-specific delivery are fermentable coating of the drug core, embedding of the drug in biodegradable matrix, formulation of drug-saccharide conjugate (prodrugs). A large number of polysaccharides have already been studied for their potential as colon-specific drug carrier systems, such as chitosan, pectin, chondroitin sulphate, cyclodextrin, dextran, guar gum, inulin, amylose and locust bean gum. Recent efforts and approaches exploiting these polysaccharides in colon-specific drug delivery system.

Keywords: Polysaccharide, biodegradable, colon.

INTRODUCTION

Many protein and peptide drugs like insulin cannot be administered through the oral route because of their degradation by the digestive enzymes of the stomach and the small intestine. Delivery of drugs to the systemic circulation through colonic absorption represents a novel mode of introducing peptides and protein drug molecules and drugs that absorb poorly from the upper gastrointestinal tract (GIT) as the colon lacks various digestive enzymes present in the upper GIT. The treatments of local diseases of colon like ulcerative colitis, Crohn's disease, and colon cancer. The various approaches used for targeting the drugs to the colon include, formation of a prodrug, multi-coating time-dependent delivery systems, coating with pH-sensitive polymers, pressure dependent systems, and the use of biodegradable polymers.

A prodrug is a pharmacologically inactive derivative of a parent molecule that requires spontaneous or enzymatic transformation within the body to release the active drug moiety. For targeting drugs to the colon, drug is to be protected from the hostile environments of the stomach and small intestine (SI). This protection in the upper GIT is affected by conjugation with carrier moieties, forming prodrugs. These prodrugs undergo enzymatic cleavage in the colon and regenerate the drug.

This prodrug when given orally is minimally absorbed in the stomach and the small-intestine and largely reaches the colon. Though these prodrugs provide site specific drug delivery, these are new chemical entities and detailed toxicological studies need to be performed before their use.

Time dependent formulations are designed to resist the release of the drug in the stomach with an additional non-disintegration or lag phase included in the formulation (which equals to the small intestinal transit time) and the release of the drug takes place in the colon. The large scale manufacturing of these systems, however, needs a lot of technological advancement and skills. Another limitation of the time dependent release systems are the variation in the gastric emptying time and small intestinal transit time[1].

The pH of the GIT is acidic in the stomach and increases in the small and large intestine. This pH variation in different segments of GI has been exploited for colon-specific delivery. Coating the drug core with pH-sensitive polymers e.g. Eudragit (methyacrylic acid-methyl methacrylate copolymers) has been successfully used for colon drug delivery. These polymers are insoluble in acidic media, but dissolves at a pH of 6 or more, thereby providing protection to the drug core in the stomach and to some extent in the SI releasing the drug in the colon. However, the pH of GIT is subject to both inter and intra individual variations, depending upon the diet, disease, age, sex and the fed/fasted state[2]. But due to the simplicity of the formulation of this device many marketed preparations utilize this approach. On prolonged use, these polymers may accumulate in the body so the use of biodegradable polymers is essential.

Osmotic systems independent of gastric residence time and metabolism by bacterial flora have also been developed for colon delivery of drugs. These systems are essentially timed release systems. OROS-CT systems[3] consist of a single or 5–6 units. These enteric coated push–pull units contain an osmotic push compartment and a drug compartment, both surrounded by a semipermeable membrane with an orifice. As the unit enters the SI, the enteric coating dissolves and the osmotic push compartment containing an osmo-polymer and an osmotic agent swells. Swelling of the osmotic push compartment forces the drug gel out of the orifice. These systems can be programmed to delay the drug release for varying durations[3].

Another strategy relies on the strong peristaltic waves in the colon that lead to a temporarily increased luminal pressure (pressure-controlled drug delivery). Pressure-sensitive drug formulations release the drug as soon as a certain pressure limit is exceeded. The pressure and the destructive force induced by peristaltic waves is certainly high in the distal part of the large intestine[4]. However, little is known about the reproducibility of this pressure and the duration of this high-pressure phase[5].

The upper part of GIT, i.e. the stomach and the duodenum has a microflora of less than 10^3-10^4 CFU/ml. These are mainly gram-positive facultative bacteria[6]. The microflora of colon on the other side is in the range of 10^{11}-10^{12} CFU/ml[7] consisting mainly of anaerobic bacteria, e.g. Bacteroides, Bifido bacteria, Eubacteria, Clostridia, Enterococci, Enterobacteria, etc.

Polysaccharides

Polysaccharides are polymers of monosaccharides (sugars). They are found in abundance, have wide availability, and are inexpensive and available in a variety of structures with a variety of properties[8]. They can be easily modified chemically and biochemically and are highly stable, safe, nontoxic, hydrophilic and gel forming and in addition biodegradable, which suggests their use in targeted drug delivery systems.

Problem encountered with the use of polysaccharides is their high water solubility. An ideal approach is to modify the solubility while still retaining their biodegradability. Large numbers of polysaccharides have already been tried for their potential as colon-specific drug carrier systems, such as chitosan, pectin, chondroitin sulphate, cyclodextrins, dextrans, guar gum, inulin, pectin, locust bean gum and amylose.

Chitosan

Chitosan is a high molecular weight, poly cationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation[9]. Chemically, it is a poly (N-glucosamine). Chitosan has favorable biological properties such as nontoxicity[10], biocompatibility[11] and biodegradability[12]. Chitosan however is soluble in dilute acid and precipitates at a pH above 7. Because of the solubility of chitosan at low pH ranges, its successful use in colon-specific delivery requires an enteric layer over the chitosan which would protect it against the acidity of the stomach. As the formulation reaches the intestine, the pH increases and the enteric layer dissolves

releasing the chitosan coated core. These cores are acted upon by microflora of the colon, degrading the chitosan and releasing the drug.

Pectins

Pectins are non-starch, linear polysaccharides extracted from the plant cell walls. They are predominantly linear polymers of mainly α (1-4)-linked D-galacturonic acid residues interrupted by 1, 2- linked L-rhamnose residues. Pectin has a few hundred to about one thousand building blocks per molecule, corresponding to an average molecular weight of about 50 000 to about 180 000.These polysaccharides remain intact in the physiological environment of the stomach and the small intestine, but are degraded by the bacterial inhabitants of the human colon[13].Being soluble in water, pectin is not able to shield its drug load effectively during its passage through the stomach and small intestine. It was found that a coat of a considerable thickness was required to protect the drug core in simulated in-vivo conditions[14]. So, the focus shifted to the development of such derivatives of pectin which were less water soluble but were degradable by the colonic microflora[15].

Guar gum

Guar gum derived from the seeds of Cyamopsistetra gonolobusis a naturally occurring galacto mannan polysaccharide. It is made up of a linear chain of β-D-mannopyranose joined by β-(1-4)linkage with α-D-galactopyranosyl units attached by 1,6-links in the ratio of 1:2.Guar gum contains about 80% galactomannan,12% water, 5% protein, 2% acid insoluble ash,0.7% ash and 0.7% fat. Guar gum hydrates and swells in cold water forming viscous colloidal dispersions or sols[16, 17, 18]. Guar gum is being used to deliver drug to the colon due to its drug release retarding property and susceptibility to microbial degradation in the large intestine[19, 20, 21].

Dextrans

Dextrans are a class of polysaccharides with a linear polymer backbone with mainly 1, 6-α-D-glucopyranosidic linkages. They are obtained from bacterial cultures of Leuconostocmes enteroides NRRL B-512. These glycosidic linkages are hydrolysed by moulds, bacteria[22, 23] and also by the mammalian cells[24]. Dextranases are the enzymes which hydrolyse these glycosidic linkages. Dextranases activity of the colon is shown by anaerobic gram-negative intestinal bacteria especially the Bacteroides[25]. Dextran has also been found to be degraded in human feaces due to bacterial action[26].Various drug-dextran prodrugs in which the drug molecule is linked to the polar dextran macromolecule remain intact and unabsorbed from the stomach and the small intestine but when the prodrug enters into the colonic microflora containing as much as 10^{11}Bacteroidesper gram[27] it is acted upon by dextranases which cleave the dextran chain randomly and at the terminal linkages releasing the drug, free into the colon.

Inulin

Inulin is a naturally occurring polysacch-aride[28] found in many plants, such as onion, garlic, chicory, artichoke. Chemically, it consists of β-2-1 linked D-fructose molecules, having a glucosyl unit at the reducing end[29]. Inulin is not hydrolysed by the secretions of the human digestive tract[30]. Bacteria present in the colon especially bifido bacteria, which constitute up to 25% of the normal gut flora in man[31] are known to ferment inulin[31].

Chondroitin sulphate

Chondroitin sulphate is a mucopolysacch-aridefound in animal connective tissues especially in cartilage. Chemically, it consists of D-glucuronicacid linked to N-acetyl-D-galactosamide. Chondroitin sulph-ate is degraded by the anaerobic bacteria of the large intestine mainly by Bacteroid esthetaiotao micron and B. o_atus[32]. Such a degradation profile suggests the use of chondroitin sulphate as a drug carrier to deliver drugs especially to the large intestine where bacteroidesare found in abundance. However, the high water solubility of chondroitin sulphate is disadvantageous.

Amylose

Amylose is a polysaccharide from plant extracts and a component of starch. It consists of D-gluco pyranose residues linked by α-(1, 4) bonds. It is a poly(1,4^{1}-α-D–glucopyranose). These naturally occurring polysaccharides possesses the ability to form films. These films are water swellable and are potentially resistant to pancreatice α-amylase[33] but are degraded by colonic bacterial enzymes[34].Amylose-Ethocel coating systems are resistant to gastric acid and small intestinal enzymes, but degradable by colonic bacteria.

Cyclodextrins

Cyclodextrins are cyclic oligosaccharides. They consist of 6–8 glucose units linked through α-1,4[1]-glucosidic bonds. Cyclodextrins are neither hydrolysed nor absorbed from the stomach and small intestine. However, in the colon the vast microflora present breaks these into small saccharides and thus are absorbed in the large intestine[35]. This property of being able to be degraded by colonic bacteria especially Bacteroidesled to its use as a colon targeting carrier.

Alginates

Alginates are a linear polymer which have 1-4 Linked β-D-mannuronic acid and α-L-guluronic acid residues arranged as blocks of either type of unit or as a random distribution of each type Alginates do not gel since they have poly (L-gluronic acids) which are rigid, Ca ions induce gelation.

Locust bean gum

Locust bean galactomannan were found to be soluble in water. Cross-linked galactomannan however led to water-insoluble film forming product showing degradation in colonic microflora[36].

Polysaccharide Nanoparticles

Nanoparticle drug delivery systems are nanometeric carriers used to deliver drugs or biomolecules. Generally, nanometeric carriers also comprise sub-micro particles with size below 1000 nm and with various morphologies, including nanospheres, nanocapsules, nanomicelles, nanoliposomes, and nanodrugs, etc. [37]. Nanoparticle drug delivery systems have outstanding advantages[38]: (1) they can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged; (2) they can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lung, spinal cord and lymph; (3) they could show controlled release properties due to the biodegradability, pH, ion and/or temperature sensibility of materials; (4) they can improve the utility of drugs and reduce toxic side effects; etc. As drug delivery system, nanoparticles can entrap drugs or biomolecules into their interior structures and/or absorb drugs or biomolecules onto their exterior surfaces. Presently, nanoparticles have been widely used to deliver drugs, polypeptides, proteins, vaccines, nucleic acids, genes and so on. Over the years, nanoparticle drug delivery systems have shown huge potential in biological, medical and pharmaceutical applications [39]. Currently, the researches on nanoparticle drug delivery system focus on:

(1) The selectness and combination of carrier materials to obtain suitable drug release speed. (2) The surface modification of nanoparticles to improve their targeting ability. (3) The optimization of the preparation of nanoparticles to increase their drug delivery capability, their application in clinics, and the possibility of industrial production

(4) The investigation of in vivo dynamic process to disclose the interaction of nanoparticles with blood and targeting tissues and organs, etc.

Polysaccharide-based nanoparticles

As for polysaccharide-based nanoparticles, Alonso et al.[40]andPrabaharan et al.[41]have ever made excellent reviews in 2001 and2005, respectively, focusing on the preparation and application of chitosan nanoparticle carriers. As time goes on, more polysaccharide based nanoparticles emerge, which greatly enriches the versatility of nanoparticle carriers in terms of category and function. According to structural characteristics, these nanoparticles are prepared mainly by four mechanisms, namely covalent crosslinking, ionic crosslinking, polyelectrolyte complexation, and self-assembly of hydrophobically modified polysaccharides.

Covalently crosslinked polysacch-aride nanoparticles

The early preparation of polysaccharide nanoparticles was by means of covalent crosslinking. Among various polysaccharides, chitosan is the early one to be used to prepare nanoparticles. As a usual crosslinker, glutaraldehyde was ever used to crosslink chitosan based nanoparticles. Recently, some chitosan nanoparticles were still crosslinked by glutaradehyde[42]. Unfortunately, the toxicity of glutaraldehyde on cell viability limits its utility in the field of drug delivery. Along with the use of biocompatible crosslinkers, biocompatible covalent crosslinking is promising. With the aid of water-soluble condensation agent of carbodiimide, natural di- and tricarboxylic acids, including succinic acid, malic acid, tartaric acid and citric acid, were used for intermolecular crosslinking of chitosan nanoparticles[43]. The condensation reaction was performed between the

carboxylic groups of natural acids and the pendant amino groups of chitosan, through which biodegradable chitosan nanoparticles were obtained. This method allows the formation of polycations, polyanions, and poly ampholyte nanoparticles. The prepared nanoparticles were stable in aqueous media at low pH, neutral, and mild alkaline conditions. In the swollen state, the average size of the particles was in the range of 270–370 nm depending on the pH.

Ionically crosslinked polysaccharide nanoparticles

Compared with covalent crosslinking, ionic crosslinking has more advantages: mild preparation conditions and simple procedures. For charged polysaccharides, low MW of polyanions and polycations could act as ionic crosslinkers for polycationic and polyanionic polysaccharides, respectively. To date, the most widely used polyanion crosslinkeris tripolyphosphate (TPP). Alonso et al.[44]first reported TPP-crosslinked chitosan nanoparticles in 1997. TPP is non-toxic and has multivalent anions. It can form a gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterions of TPP[45]. From then on, TPP-chitosan nanoparticles have been widely used to deliver various drugs and macromolecules[46-55].

Polysaccharide nanoparticles by polyelectrolyte complexation (PEC)

Polyelectrolyte polysaccharides can form PEC with oppositely charged polymers by intermolecular electrostatic interaction. Polysaccharide-based PEC nanoparticles can be obtained by means of adjusting the MW of component polymers in a certain range. In theory, any polyelectrolyte could interact with polysaccharides to fabricate PEC nanoparticles. However, in practice, these polyelectrolytes are restricted to those water-soluble and biocompatible polymers in view of safety purpose. In this sense, chitosan is the only natural polycationic polysaccharide that satisfies the needs. There are many negative polymers complexed with chitosan to form PEC nanoparticles, which can be divided into polysaccharides, peptides, polyacrylic acid family.

Negative polysaccharides

Cui et al.[56]used carboxy methyl cellulose to complex chitosan to form stable cationic nanoparticles and investigated the topical application of these nanoparticles containing plasmid DNA as a potential approach to genetic immunization. Plasmid DNA was coated on pre-formed cationic chitosan/carboxy methyl cellulose nanoparticles. Selected plasmid DNA-coated nanoparticles (with plasmid DNAmup to 400 mg/ml) were stable to challenge with serum. Several different chitosan-based nanoparticles containing plasmid DNA resulted in both detectable and quantifiable levels of luciferase expression in mouse skin 24 h after topical application and significant antigen-specific IgG titer to expressed ß-galactosidase at 28 days. Chen et al.[57]developed chitosan/dextran sulfate nano particle delivery system by employing a simple coacervation process. The study investigated the effect of the weight ratio of the two polymers on particle size, surface charge and entrapment efficiency, and release characteristics of anti-angiogenesis peptide. Particles of 223 nm mean diameter were produced under optimal conditions with a zeta potential of approximately -32.6 mV. The physicochemical and release characteristics of the nanoparticles could be modulated by changing ratios of two ionic polymers. Tiyaboonchai et al.[58]developed a nanoparticulate delivery system for amphotericin B with chitosan and dextran sulfate together with zinc sulfate as a crosslinking and hardening agent.

Negative peptides

Lin et al.[59]prepared poly-γ-glutamic acid/chitosan nanoparticle system using ionic-gelation method. Evaluation of the prepared nanoparticles in enhancing intestinal paracellular transport was investigated in vitro in Caco-2 cell monolayers. It was found that the nanoparticles with chitosan dominated on the surfaces could effectively reduce the transepithelial electrical resistance of Caco-2cell monolayers and opened the tight junctions between Caco-2 cells and allowed transport of the nanoparticles via the paracellular pathways. Moreover, the nanoparticles were further used for transdermal gene delivery. As compared with chitosan/DNA, chitosan/poly-γ-glutamic acid/DNA improved their penetration depthinto the mouse skin and enhanced gene expression. These observations may be attributed to the fact that chitosan/poly-γ-glutamic acid/DNA were more compact in their internal structures and had a greater density than their chitosan/DNA counterparts, thus having a larger momentum to penetrate into the skin barrier[60].

Polyacrylic acid family

Sajeeshet al.[61] prepared pH sensitive poly methacrylic acid/ chitosan/polyethylene glycol nanoparticles under mild aqueous. Free radical polymerization of methacrylic acid was carried out in presence of chitosan and polyethylene glycol using a water-soluble initiator and particles were obtained spontaneously during polymerization without using organic solvents or surfactants/steric stabilizers. Insulin and bovine serum albumin as model proteins were incorporated into the nanoparticles by diffusion filling method and their in vitro release characteristics were evaluated at pH 1.2 and 7.4. The nanoparticles exhibited good protein encapsulation efficiency and pH responsive release profile was observed under in vitro conditions. Chen et al.[62]reported the formation of chitosan/poly (acrylic acid) nanoparticles.

Self-assembly of hydrophobically modified polysaccharides

When hydrophilic polymeric chains are grafted with hydrophobic segments, amphiphilic copolymers are synthesized. Upon contact Poly (isobutyl Cyanoacrylate) with an aqueous environment, polymeric amphiphiles spontaneously form micelles or micelle like aggregates via undergoing intra- or-intermolecular associations between hydrophobic moieties, primarily to minimize interfacial free energy. These polymeric micelles exhibit unique characteristics, depending on hydrophilic/hydrophobic constituents, such as unusual rheological feature, small hydro dynamic radius (less than micro size) with core-shell structure, and thermodynamic stability. In particular, polymeric micelles have been recognized as a promising drug carrier, since their hydrophobic domain, surrounded by a hydrophilic outer shell, can serve as a preservatory for various hydrophobic drugs[63]. In recent years, numerous studies have been carried out to investigate the synthesis and the application of polysaccharide-based self-aggregate nanoparticles as drug delivery systems.

Linear hydrophobic molecules

Poly (ethylene glycol) has been employed extensively in pharmaceutical and biomedical fields because of its outstanding physic chemical and biological properties including hydrophilic property, solubility, non-toxicity, ease of chemical modification and absence of antigenicity and immunogenicity. Therefore, poly (ethylene glycol) has been often used as a soluble polymeric modifier in organic synthesis; it is also widely used as a pharmacological polymer with high hydrophilicity, biocompatibility and biodegradability. It is ideal for prevention of bacterial surface growth, decrease of plasma protein binding and erythrocyte aggregation, and prevention of recognition by the immune system. In recent years, poly(ethylene glycol)[64]. Yoksanet al.[65] grafted poly (ethylene glycol) methyl ether onto N-Phthaloyl chitosan chains.

Cyclic hydrophobic molecules

Cholesterol is an indispensable lipid in animals, which not only involve in the formation of cell membranes but also works as a raw material for the synthesis of bile acids, vitamin D and steroid hormones. Conjugating hydrophobic cholesterol to hydrophilic polysaccharides may form amphiphilic copolymer which may further form self-assembly nanoparticles in aqueous solution. Wang et al.[66] synthesized cholesterol-modified chitosan conjugate with succinyl linkages. The CMC was 1.16×10^{-2}mg/ml in 0.1 M acetic acid solution. The conjugates formed monodisperse self-aggregated nanoparticles with a roughly spherical shape and a mean diameter of 417.2 nm by probe sonication in aqueous media.

PERSPECTIVE

As reviewed above, so many nanoparticle drug delivery systems have been prepared. It can be predicted that, more nanoparticle drug delivery systems will emerge. Until now, these nanoparticles are generally investigated in terms of their physicochemical properties, drug-loading ability, in vitro toxicity, and comparatively simple in vivo tests. The more important issues, such as the specific interaction of these nanoparticles with human organs, tissues, cells, or biomolecules, the effect on human's metabolism brought by the nanoparticles and the wider application of these nanoparticles for drug delivery, etc. await further deep study, which will be focused on in the near future.

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