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Review

Lipid-based nanosuspensions for oral delivery of peptides, a critical review

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ABSTRACT

Peptides are therapeutic molecules that can treat selectively and efficiently a wide range of pathologies. However, their intrinsic properties cause their rapid degradation in the human gastrointestinal (GI) tract resulting in poor bioavailability after oral administration. Yet, their encapsulation in nanocarriers offers them protection from this harsh environment and increases their permeability across the epithelium border. In particular, Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) have proven to improve peptide oral bioavailability.

This article details different techniques used to produce SLN and NLC with potential or effective peptide encapsulation. Basic principles of covalent and non-covalent lipidization are described and discussed as a prerequisite to improve hydrophilic peptide encapsulation in lipid-based nanosuspensions. The last part of this review provides the key evaluation techniques to assay SLN and NLC for peptide oral bioavailability enhancement. Methods to assess the protective effects of the carriers are described as well as the techniques to evaluate peptide release upon lipid digestion by lipases. Furthermore, this review suggests different techniques to measure permeability improvements and describes the main *in vitro* cell models associated.

1. Introduction

Therapeutic peptides and proteins are a new class of promising pharmaceutical ingredients that can treat a wide variety of affections with effective and potent action. Indeed, in 2008, > 130 different proteins or peptides have been approved for clinical use by FDA (Muheem et al., 2016). In 2013, this market was estimated to represent more than $40 billion per year (Craik et al., 2013). This success comes from their composition and structure that enable very specific binding to therapeutic site hence increasing their efficiency and limiting side effects (Craik et al., 2013). In addition, they accumulate less in tissues and are supposed to be less toxic than classic chemical drug molecules (Craik et al., 2013; Morishita and Peppas, 2006). Most of the therapeutic peptides are administered parenterally and more specifically via intravenous route. Since they exhibit a very short half-life in human physiological fluids, this operation must be repeated frequently leading to real annoyance for patients (Almeida and Souto, 2007; Brayden and O'Mahony, 1998; Gupta et al., 2013). Development of new dosage forms enabling alternative routes of administration is being widely investigated. Oral route is the preferred way of administration for patients. However, intrinsic properties of peptides generate numerous biopharmaceutical issues leading to low oral bioavailability (< 1%) (Brayden and O'Mahony, 1998). Nevertheless, in 2016, 8 peptides where already on the market for oral administration, among which 4 are systemically absorbed (Cyclosporin A, Desmopressin, Taltirelin and Glutathione) while the 4 others are intended for local intestinal effect (Linaclotide, Vancomycin, Colistin and Tyrothricin) (Aguirre et al., 2016). In order to design an efficient drug delivery system for oral peptide delivery, some physical, chemical and biological barriers should be overcome. The main barriers limiting the oral bioavailability of peptides are the acidic pH of the stomach, the degradation by proteases and peptidases, the reduction by glutathione, and the difficulty to cross the mucus and intestinal epithelium.

The first barrier limiting peptide oral bioavailability is the stomach where the acid pH (2–3 in fasted condition) can cause denaturation of peptides. Indeed, below pH 3, acid-catalyzed hydrolysis of asparagine

Abbreviations: AOT, dioctyl sulfoccinate; CEH, cholesteryl ester hydrolase; CPP, cell penetrating peptide; DL, drug load; EE, encapsulation efficiency; FA, Fatty Acid; GI, Gastrointestinal; GSH, reduced glutathione; HIP, hydrophobic ion pairing; HPH, high pressure homogenization; LHR, luteinizing hormone-releasing hormone; MDCK, Mardin-Darby canine kidney; NLC, nanostructured lipid carrier; PDI, polydispersity index; PEO, polyethoxylated; PIT, phase inversion temperature; REAL, reversible aqueous lipidization; RESS, rapid expansion of SCF solution; SAS, supercritical fluid anti-solvent; SCF, supercritical fluid; sCT, salmon calcitonin; SEDDS, self-emulsifying drug delivery system; SNEFDS, self-nanoemulsifying drug delivery system; SLN, solid lipid nanoparticle; TEER, transepithelial electric resistance

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and glutamine can lead to peptide deamination (Manning et al., 1989; Reubsaet et al., 1998). Furthermore, in very acidic medium (pH < 4), peptides are fully ionized which can lead to intrinsic electrostatic repulsions and potential loss of conformation resulting in loss of biological activity. In addition, the stomach secretes pepsinogen that transforms into pepsin – an aspartic peptidase – that cleaves bonds between hydrophobic amino acids and facilitates the action of proteases located in the small intestine (Malhaire et al., 2016; Rao et al., 1998).

Then the bolus reaches the duodenum where is located the main enzymatic barrier to peptide oral bioavailability. Duodenum exhibits a pH of 5–6 and is the receptor of bile and pancreatic juice secretions containing proteases: trypsin, α-chymotrypsin, elastase, exopeptidases, carboxypeptidases A&B (serine proteases) (Leonaviciute and Bernkop-Schnürch, 2015). These enzymes break peptides into smaller ones and free amino acids by nucleophilic attacks on specific bonds with different binding sites for each enzyme (Malhaire et al., 2016). Peptides displaying a disulfide bond can also be inactivated when subjected to thiol-disulfide exchange reaction with reduced glutathione (GSH) (Schmitz et al., 2006). Endopeptidases, carboxypeptidases and aminopeptidases, present at the brush border membrane add a supplementary barrier to absorption of intact peptides (Pereira de Sousa and Bernkop-Schnürch, 2014).

The human intestinal epithelium is designed to limit the absorption of pathogens in the systemic circulation and acts as the next and major barrier to peptide oral bioavailability. Three main pathways can be considered for peptide absorption depending on their specific physicochemical properties: transcellular, paracellular and receptor-mediated transports. The epithelium border is in great majority composed of enterocytes with lipophilic membranes. Peptides being generally highly hydrophilic molecules are then poor candidates for the transcellular pathway, except for some lipophilic peptides such as Cyclosporine A. These epithelium cells are separate by tight junctions allowing the transport of small hydrophilic molecule toward *lamina propia*. This paracellular pathway is limited for peptides whose size was arbitrary fixed under 50 amino acids (Craik et al., 2013; Li et al., 2012). The junctional space between cells displays a negative electrostatic field that may alter permeability of some molecules by charge-charge interactions. Active-transportation across enterocytes is only possible for small peptides (Pawar et al., 2014). In particular, PepT1 transporter limits absorption to di and tripeptides (Miner-Williams et al., 2014). Finally, the intestinal epithelium is covered by mucus. This viscoelastic gel can interact with peptides and prevent their permeation across the epithelium.

All the physiological barriers described above have been known for a long time and have urged formulators to develop strategies to overcome them. These strategies comprise the use of enteric protection, enzyme inhibitors, permeation enhancers, mucoadhesive polymers, the modification of the therapeutic peptide structure, or the use of advanced drug delivery systems.

Fragile molecules can be easily protected for acidic environment by using enteric protection (coating or capsules). They are generally composed of cellulosic or acrylic based-polymers that are very stable at acidic pH, in the stomach, and fast dissolving when pH increases. Thus, they release their content in the upper part of the small intestine where peptides are less susceptible to pH-induced degradation (Bruno et al., 2013; Gao et al., 1998; Leonaviciute et al., 2016). Enteric protection also prevent degradation from gastric enzymes and provide a gradual and steady drug release upon dissolution (Leonaviciute and Bernkop-Schnürch, 2015).

Proteases inhibitors can temporarily restrict the activity of proteolytic enzymes (Bernkop-Schnürch, 1998). Their use is nevertheless questioned because their action is not specific to therapeutic peptides and can disturb the metabolism of dietary proteins (Morishita and Peppas, 2006). Furthermore, repeated administration of these substances can induce an over-secretion of enzymes leading to pancreatitis overtime.

Permeation enhancers alter the natural anatomy of the intestinal membrane by changing mucus viscosity, increasing intestinal membrane fluidity or modifying structural integrity (Pawar et al., 2014). Despite the interesting results obtained by addition of permeation enhancers, one has to keep in mind that their use not only facilitates the passage of the drug but also of unwanted substances. Thus, their repeated use, in chronical diseases for example, has to be carefully considered before including them in dosage forms.

Mucoadhesive systems prolong residence time of biomolecules at the absorption site and enable an increased concentration gradient between the system and the intestinal membrane. Several polymers have been reported to allow adhesion of nanocarriers transporting peptides and proteins such as PEG, cyclodextrins, poly (ethyl cyanoacrylate) or polyallylamine (Gupta et al., 2013). Chitosan-based nanoparticles are one of the most used systems. Chitosan is a cationic polymer which allows electrostatic interactions with the negatively charged sialic acid residues of the mucosal surface (Mrsny, 2012). Thiolated polymers have also been widely studied due to their ability to form strong covalent bond with cysteine-rich subdomains of the mucus glycoprotein (Muheem et al., 2016). However, mucoadhesive systems are eliminated upon mucus degradation and regeneration. The intestinal epithelium is the most rapidly renewed body tissue with a complete renewal every 4–5 days (Brayden et al., 2015; Naudi, 2012).

Chemical conjugation of peptides with PEG (PEGylation) have also been reported as one of the most successful technique to increase peptides residence time by limiting their interaction with mucin. Furthermore, their long chains are able to increase solubility of the biomolecules while shielding them from enzymatic degradation. Peptides can be absorbed via associated transport mechanisms using membrane transporters or receptor mediated endocytosis by grafting specific ligands to macromolecules. Most reported ligands are cell-penetrating peptides (CPPs). CPPs are small peptides, capable of internalizing bioactive molecules into cells by perturbation of the lipid bilayer of enterocytes (Brooks et al., 2005).

As a minimum lipophilicity is required to cross the epithelial barrier through transcellular way, some trials were conducted to increase lipophilicity of biomolecules. This lipidization operation consists either in the covalent grafting of a hydrophobic moiety or the non-covalent interaction with a hydrophobic compound, thus enhancing the hydrophobicity of the peptides (Morishita and Peppas, 2006). Yet, to ensure a preservation of the peptide therapeutic efficacy, this operation has to be reversible once the epithelial membrane has been crossed. Increasing lipophilicity has also showed an increase association with chylomicrons, making them able to undergo a significant lymphatic transport. Yet, chemical modification of the active substances raises the question of therapeutic efficacy of peptides *in vivo* if structural modifications affect their capacity to bind with the receptor sites (Meyer and Manning, 1998).

Finally, advanced drug delivery systems can be used both to enhance protection of peptides against the harsh environment of the GI tract and to increase transport across the epithelial border. These vectors can be hydrogels, micelles, microspheres, and polymeric and/or lipid-based nanoparticles. Among them, nanosized carriers are of great interest as their dimension favors the transport across the epithelial border and are supposed to provide a better drug distribution at the apical surface of the intestinal epithelium compared to other solid dosage forms (Mrsny, 2012). When drugs are attached to carriers, their fate *in vivo* is determined by the carrier system properties (Almeida and Souto, 2007). Lipid-based nanocarriers are promising tools to deliver peptides as lipids are GRAS, biodegradable excipients and consequently do not accumulate in tissues. Furthermore, lipid-based molecules are known to increase transcellular transport by transient disruption of cells lipophilic bilayers. Furthermore, some of the lipid digestion products are known to alter tight junctions with a consequent increase of drug transport through the paracellular pathway (Niu et al., 2016). Numerous lipid-based nanocarriers are reported in the literature: