Journal of Controlled Release 255 (2017) 242–257

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Lipophilic peptide character – What oral barriers fear the most

Ožbej Zupančič, Andreas Bernkop-Schnürch*

Department of Pharmaceutical Technology, Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Innrain 80/82, Center for Chemistry and Biomedicine, 6020 Innsbruck, Austria

ARTICLE INFO

Keywords:
Lipophilicity
Hydrophobic ion pairing
Real
Cyclization
Esterification
Presystemic metabolism
Intestinal permeation

ABSTRACT

Peptide therapeutics is currently one of the fastest growing markets worldwide and consequently convenient ways of administration for these drugs are highly on demand. In particular, oral dosage forms would be preferred. A relative large molecular weight and high hydrophilicity, however, result in comparatively very low oral bioavailability being in most cases below 1%. Lipid based formulations (LBF), in particular self-emulsifying drug delivery systems (SEDDS) and solid lipid nanoparticles (SLN) as well as liposomes are among the most promising tools for oral peptide delivery. Key to success in orally delivering peptides via LBF seems to be a sufficiently high lipophilic character of those therapeutic agents. Hence, different non-covalent and covalent peptide lipidization methods from drug delivery point of view are presented. On the one hand, among non-covalent lipidization methods hydrophobic ion pairing seems to be a promising way to sufficiently increase peptide lipophilicity providing high drug payloads in the lipid phase, a protective effect against presystemic metabolism via thiol-disulphide exchange reactions and proteolysis as well as an improved intestinal membrane permeability. On the other hand, covalent methods like conjugating fatty acids via amidation, esterification, reversible aqueous lipidization (REAL) and cyclization also show potential. The present review therefore describes those lipidization methods in detail and critically evaluates their contribution in successfully overcoming the oral barriers.

1. Introduction

Recent advances in biotechnology have led to rapid increase in synthesis and commercialization of numerous peptide drugs [1]. Currently > 600 peptide drugs are in preclinical or clinical trials, giving the global peptide market a high growth potential [2,3]. On the one hand, peptides have advantages over conventional small molecule drugs including high potency, selectivity, excellent safety profile, tolerability and fewer side effects [1,4]. On the other hand, the majority of them have to be administered via injections being related with pain, discomfort and consequently low patient compliance [1]. Oral peptide delivery is indeed an ongoing challenge, which is evident from numerous published strategies over the past decades [3,5]. Comparing the data of so far available in vivo studies and clinical trials, delivering peptides via LBF such as (micro)emulsions including SEDDS [6–12], SLN [13–20] and liposomes [21–33] is likely one of the most promising strategies. Increasing intestinal membrane permeability, modulation of tight junctions and reducing proteolytic degradation are just some of the benefits when utilizing lipid excipients in oral peptide delivery [3]. However, only few oral peptide formulations containing lipophilic auxiliary agents have up to date reached clinical trials or commercialization [34,35]. The only commercialized oral peptide LBF Sandimmune Neoral® contains cyclosporine dissolved in lipid preconcentrate, forming an o/w emulsion under in vivo conditions, where the peptide remains incorporated in the lipid matrix of LBF. Following this example, this review will focus therefore just on one important aspect having a substantial impact on the success of oral peptide delivery strategies – namely the lipophilic character of the therapeutic peptide.

2. Lipidization

2.1. Hydrophilic character of peptides and its consequences

Peptide chemical structure has two fundamental disadvantages when it comes to oral administration – relative high molecular weight and high hydrophilicity [35–40]. The former restricts peptides to paracellular absorption, which comprises of a very small percentage of the total epithelial surface area [37,41–43]. The latter, on the other hand, prevents the therapeutic peptides from permeating the phospholipid bilayer of epithelial cell membranes as they are forced to break its hydrogen bonds with solvating water in order to interact with the lipid

* Corresponding author.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

http://dx.doi.org/10.1016/j.jconrel.2017.04.038
Received 19 February 2017; Received in revised form 21 April 2017; Accepted 25 April 2017
Available online 28 April 2017

bilayer [37,43–45]. As a consequence of this low membrane permeability systemic uptake via the passive transcellular route is strongly limited. Thus, the key factor in increasing peptide lipophilicity is the reduction of its hydrogen bonding potential [44]. Furthermore, orally administered peptides containing disulphide bonds may interact in the intestine with endogenous glutathione and thiol substructures of food via thiol-disulphide exchange reactions leading to peptide conformational changes and subsequently their inactivation [46,47]. Moreover, an insufficient lipophilic character exposes peptides to harsh presystemic metabolism by intestinally secreted trypsin, α-chymotrypsin, elastase, carboxypeptidases A and B as well as membrane bound peptidases leading to their inactivation [48–51]. Additionally, polar functional groups of peptides lead to ionic interactions and hydrogen bonding with intestinal content and mucus contributing to a low oral bioavailability and high variability in drug uptake [44]. Indeed, the mucus layer covers the luminal surface of the gastrointestinal tract and is composed up to 95% of water as well as cross-linked and entangled mucin fibres, carbohydrates, DNA, lipids, salts, antibodies, bacteria and cellular debris [52]. As such, mucus acts as a filter for charged hydrophilic peptides either by size exclusion mechanism or ionic peptide-mucin interactions, resulting in poor peptide mucus permeation [53]. However, there are two opposing strategies to improve peptide diffusion through mucus – the mucoadhesive and the mucodiffusion principle [52]. Firstly, incorporating peptides in mucoadhesive polymers and their thiolated counterparts was shown to facilitate peptide diffusion through mucus layer due to close contact of polymer matrix with absorption membrane, providing steep concentration gradient. Moreover, polymer matrix can protect peptides against proteolysis in GIT as well as offers controlled drug release [3,54]. On the contrary, mucodiffusive LBF with neutral or negatively charged surface show even greater potential [3]. The hydrophilic PEG surface corona of LBF enhances their mucus diffusion and also prevents their interaction with biological enzymes and mucin [3]. From oral peptide delivery point of view, it would be highly beneficial if LBF contain the peptide drug in its lipid matrix. Accordingly, a high lipophilic character of orally given peptides seems to be essential.

2.2. How to measure lipophilicity

Lipophilicity is defined as the affinity of a molecule or moiety for a lipophilic environment. Indeed, as from the drug delivery point of view it is advantageous to incorporate therapeutic peptides in lipophilic carrier systems such as SEDDS, SLN and liposomes, the determination of their solubility in lipophilic excipients used in these formulations is essential. Lipophilicity is commonly measured by peptide distribution behaviour in a biphasic system, which can be liquid-liquid or solid-liquid. On the one hand, the gold standard for direct liquid-liquid phase lipophilicity characterization of neutral substances is the octanol/water partitioning coefficient (log P) [55,56]. However, since most of peptides are ionized, distribution coefficient (log D) expressing the contribution of all neutral and ionized species at given pH, is utilized. For simplicity reasons, in the scope of this review, log P values of peptides are compared. Octanol is used due to its structural similarity with phospholipids in the cell membrane. However, octanol is also a known hydrogen bond donor/acceptor and therefore alternative solvents with different hydrogen bond properties such as heptane, chloroform, cyclohexane and propylene glycol dipelargonate have been proposed [57,58]. One the other hand, since chromatographic retention of substances in a reversed phase column and *n*-octanol/water partitioning are energetically analogous, reversed phase chromatography is becoming the preferential indirect solid-liquid phase method for determining log P [56,59]. Octadecyl-bonded silica (C-18 RP-HPLC) and immobilized artificial membranes mimicking the phospholipid bilyer (IAM HPLC) are normally used as stationary phases. Here, a prolonged retention time of lipidized peptide is observed with respect to the native peptide [60–63]. From difference in retention times the representative lipophilicity factor, log k_w, which is directly related to log P, can be calculated [56]. Noteworthy, peptide lipophilicity can be also determined by potentiometric titration, where the peptides are partitioned between liposomal membrane and water and the dissociation constants (pKa) are determined by adding high precision titrators [56]. In addition, fast and simple indirect methods to measure lipophilicity include thin layer chromatography (TLC) [56] and various computational methods [64].

2.3. Covalent vs. non-covalent lipidization

Lipidization not only alters peptides lipophilicity, but it also may have a substantial impact on its secondary structures, self-assembling properties and its ability to bind to target receptors. Moreover, a significant change in ADME properties including metabolic and plasma stability, membrane permeability and bioavailability can be expected [55]. In some cases, these changes may cause partial or total loss of the peptides biological activity [61,65,66]. From the commercial point of view, non-covalent lipidization is preferred. On the one hand, non-covalent lipidization offers a straightforward lipophilicity increase excluding intensive labour with multiple reactions and purification steps. Additionally, non-covalent interactions enable native peptide regeneration in vivo, restoring its secondary and tertiary structures and thus preserving its affinity to target receptors. Moreover, the peptide might not be regarded as a new active pharmaceutical ingredient (API) as it would definitely be the case for all covalently lipidized peptides. On the other hand, the shortcoming of such labile non-covalent bonds is the dissociation of peptide-complex in vivo before it reaches the desired target [67–69]. In such cases, reversible covalent lipidization methods, where the parent peptide is regenerated in vivo, is recommended. In the following chapters, different strategies to increase the lipophilic character of peptide drugs including non-covalent and covalent lipidization approaches as illustrated in Fig. 1 are described.

3. Non-covalent lipidization

Peptides can interact non-covalently with lipidization agents through hydrogen bonding, hydrophobic interactions, complexation with divalent metal ions and ionic interactions, forming water insoluble complexes. A typical example of hydrogen bonding and hydrophobic interactions are tannines. For instance, it was shown in the previous studies that protein-tannin complexes are stable at gastrointestinal pH and in the presence of proteolytic enzymes such as pepsin, trypsin, α-chymotrypsin, elastase and carboxypeptidase A and B as well as bile salts [70,71]. Furthermore, metal ions such as Zn^{2+}, Cu^{2+} or Ni^{2+} are able to form coordinative complexes with peptides, which cause conformational changes and stabilize the peptide structure [72–74]. Oxytocin, for example, undergoes a conformational change when coordinated with Cu^{2+} or Zn^{2+}. This causes the carbonyl oxygens of the ring to be directed towards the core reducing the peptides hydrogen bonding potential and rendering its surface more hydrophobic [73,75]. Also, Zn^{2+} is well known to coordinate with peptides, forming water insoluble complexes. In previous studies, various water insoluble zinc-peptide complexes such as with insulin [76–78], hirudin [79,80], dalarein [81,82], buserelin [83], gonadotropin-releasing hormone [81], thyrotropin-releasing hormone [81], adrenocorticotropic hormone [81] and thyroliberine [82] have been prepared. However, the most efficient approach for non-covalent peptide lipidization seems to be via ionic interactions, where peptide lipophilicity is increased by peptide net charge neutralization by anionic, amphoteric or cationic surfactants.

3.1. Interactions between peptides and surfactants

Peptide-surfactant interactions are a complex process, dependent on peptides primary structure, pH, ionic strength, temperature, surfactant