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Review Article

Theme: Preclinical Peptide Developability Assessment
Guest Editors: Annette Bak and Weiguo Dai

Strategic Approaches to Optimizing Peptide ADME Properties

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Abstract. Development of peptide drugs is challenging but also quite rewarding. Five blockbuster peptide drugs are currently on the market, and six new peptides received first marketing approval as new molecular entities in 2012. Although peptides only represent 2% of the drug market, the market is growing twice as quickly and might soon occupy a larger niche. Natural peptides typically have poor absorption, distribution, metabolism, and excretion (ADME) properties with rapid clearance, short half-life, low permeability, and sometimes low solubility. Strategies have been developed to improve peptide drugability through enhancing permeability, reducing proteolysis and renal clearance, and prolonging half-life. *In vivo*, *in vitro*, and *in silico* tools are available to evaluate ADME properties of peptides, and structural modification strategies are in place to improve peptide developability.

KEY WORDS: ADME; peptides; pharmacokinetics; proteolysis; renal clearance.

INTRODUCTION

The human genome project indicated there are 30,000 human genes (1). Among them, ~3,000 are disease-modifying genes and ~3,000 are druggable genes (1). This suggests that only 2–5% of the human genome are small molecule drug targets (~600–1,500), which presents a great opportunity to use peptides or large molecules to treat human diseases.

Development of peptides into drugs has long been recognized as an important opportunity in order to address certain disease targets that would otherwise be challenging using small molecules. In the 1980s, huge investments were channeled into the development of peptide-based drugs, only to realize the nondrug-like nature of this structure class (2). Subsequently, many drug developers and investors dismissed peptides as potential drugs.

With the advances in genomics, transcriptomics, and proteomics, many new disease targets feature protein–protein interactions with shallow binding pockets covering wide surface area. Peptides and proteins are more suitable for these types of disease targets than small molecules. Personalized medicine and greater emphasis on efficacy and safety in the current regulatory environment require therapeutic agents to have high versatility, specificity, and safety. Peptides have greater potential to meet the ever-increasing expectations of new drugs, as they are highly specific to individual protein targets, amenable to site-specific modification and highly selective. In addition, the advances in recombinant protein expression technologies, the development of more efficient and economic peptide synthesis, the improvement of peptide purification systems and new analytical tools have been essential for the revival of the peptide field in the recent decade. Peptides have the potential to offer the advantages of both small molecule drugs and proteins.

Unlike small molecule drugs, peptides represent a very small portion (2%) of the worldwide drug market (3). The annual sales of peptide drugs are about $20 billion (3). However, peptide drugs can be quite successful with several blockbuster peptide drugs on the market, *e.g.*, Copaxone, Lupron, Zoladex, Sandostatin, and Velcade (4–6). Six new peptides received first marketing approval as new molecular entities in 2012 (6). Most peptide drugs (~85%) are synthetically made, which is largely credited to the development of solid-state peptide synthesis, and a small number (~15%) are prepared using recombinant technology. With the use of unnatural amino acids and pseudo-peptide bonds, chemical synthesis offers more diversity and patentability than peptides derived from recombinant technology (7). There are currently ~70 approved peptide drugs on the market, ~200 in clinical development, and ~600 in the preclinical drug discovery stage (Fig. 1) (3). The peptide market is growing twice as quickly as the rest of the drug market, suggesting peptides might soon occupy a larger niche (7,8).

Peptide drugs cover a wide range of therapeutic areas, such as diabetics, cancer, osteoporosis, hormone therapy, cardiovascular diseases, anemia, bowel syndrome, Cushing's disease, multiple sclerosis, HIV, and many more (6,9). The focus of peptide drugs is shifting from hormone therapy and diagnosis to cancer and infection.

Ranging in size between small molecules and proteins, peptides present a unique opportunity and challenge for the

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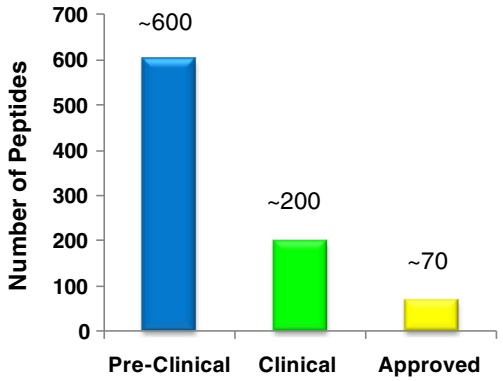

Fig. 1. Stages of peptide drugs in discovery and development (3)

pharmaceutical industry compared to small molecules (Table I) (9,10). Small molecules represent ~80% of the drug market and peptides only represent ~2%. Small molecules are usually cheaper and relatively easier to synthesize. Peptides are typically more difficult to make and have synthetic and production cost about 10–100-fold higher than small molecules (7,11). Though some small molecules also face similar challenges, many of them are permeable through cell membranes, stable, and have good oral bioavailability. Peptides usually have low cell membrane permeability, limited stability, poor oral bioavailability, and are usually administrated by injection [*e.g.*, subcutaneous (SC), intramuscular (IM), intravenous (IV)]. Unmodified peptides tend to have short half-lives and are typically limited to extracellular targets due to poor cell membrane penetration. With all these challenges, why are we interested in developing peptide drugs? Peptides have unique properties that are very appealing as therapeutic agents, such as high binding affinity toward therapeutic targets, easier to identify as the mechanisms of action are well-defined, excellent target specificity, broad coverage of disease targets, low toxicity and immunogenicity, minimal risk of drug-drug interaction potential, and low impact of generic erosions (3,5,6,12). Therefore, peptides are an area of high interest for many drug developers.

Table I. Comparison Between Peptides and Small Molecules

Small molecules	Peptides
• ~80% drug market	• ~2% drug market
• Low cost	• High cost
• Permeable	• Low permeability
• Stable	• Limited stability
• Good oral bioavailability	• Poor oral bioavailability
	• More challenging synthesis
• Easier synthesis	• Short half-life
	• Limited to extracellular targets
	• High binding affinity
	• Easier to identify
	• Excellent target specificity
	• Broad disease targets
	• Mechanism of action well understood
	• Low toxicity and immunogenicity
	• Low risk of drug–drug interaction potential
	• Lower impact from generics

ADME CHALLENGES OF PEPTIDES

It is often challenging for peptides to become successful drugs due to multiple absorption, distribution, metabolism, and excretion (ADME) issues such as low permeability, metabolic instability, short half-life, and limited residence time in tissues. Most peptides have low cell membrane permeability owing to high hydrogen bonding capacity and low lipophilicity (13). Low oral bioavailability of peptides is mostly caused by low absorption and high first-pass extraction due to enzymatic- and pH-mediated hydrolysis in the gastrointestinal (GI) tract and liver (14). Consequently, peptide drugs are frequently administered as injectables or through other alternative delivery routes, such as inhaled, buccal, intranasal, and transdermal. Unmodified peptides usually have very short half-lives (*e.g.*, minutes) resulting from extensive proteolysis in blood, kidneys, or liver and/or rapid renal clearance (15). *In silico*, *in vitro*, and *in vivo* tools have been developed to address the ADME challenges of peptides. Structural modification strategies are in place to enhance peptide developability (5,12,16,17).

Absorption

With a few exceptions (*e.g.*, cyclosporine A), most peptides have less than 1% oral bioavailability (18). They tend to show high inter-subject and inter-species variability in plasma exposure (19). In spite of the major hurdles to achieve oral bioavailability, there is still significant effort in developing oral peptides owing to their high market potential and patient compliance (20,21). Examples of oral peptides in development include calcitonin in phase III, glucagon-like peptide-1 (GLP-1), and parathyroid hormone in phase I (5,12,15).

Following SC or IM delivery routes, peptides enter systemic circulation either through blood capillaries (molecular weight (MW) <1 kDa) or lymphatic vessels (MW >16–22 kDa) (15,22). For most therapeutic peptides with typical MW of 1–10 kDa, combined absorption through both blood and lymphatic systems is expected with the diffusion-driven uptake into blood capillaries being the predominant pathway (15). "Flip-flop" pharmacokinetics (PK) (absorption constant is much slower than elimination constant) may occur when absorption is slower than elimination leading to prolonged half-life and pharmacodynamic (PD) effect (15). An example is the gonadotropin-releasing hormone (GnRH) agonist leuprolide, where a long-acting monthly IM depot injection releases the peptide slowly and continually into blood to provide long-term suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) for the treatment of prostate cancer and endometriosis (23).

Permeability Methods for Peptides

Peptides can be absorbed by (a) passive diffusion through the lipid membrane, (b) paracellular pathway, and (c) transporter-mediated processes [*e.g.*, peptide transporter 1 (PEPT1), sodium-dependent multivitamin transporter (SMVT), vitamin B_{12} transport system] (12). Small molecule *in vitro* permeability and transporter assay platforms are largely applicable to study peptide permeability and

transporter characteristics, such as log *D* (16,24), parallel artificial membrane permeability assay (PAMPA) (13,25), Madin-Darby canine kidney (MDCK) (26,27), Caco-2 (28–30), PEPT1 (31–33), and SMVT (34). The Caco-2 cell line possesses many of the human intestinal transporters (*e.g.*, PEPT1, SMVT) and can be used to identify peptides with high absorption potential not only by transcellular or paracellular passive diffusion mechanisms but also by active uptake processes (30). The challenges of these assays for measuring peptide permeability are as follows: (1) high nonspecific binding to assay plates, pipette tips, and transwell filter membranes and (2) degradation mediated by enzymes expressed in the cell systems or by pH-mediated hydrolysis. To minimize the impact of nonspecific binding, low binding tips and plates are typically used, which are commercially available (http://www.labonline.com.au/products/48460-Eppendorf-LoBind-Tubes-and-Plates-and-epT-I-P-S-LoRetention-pipette-tips). Serum proteins [*e.g.*, bovine serum albumin (BSA)] are often added to the receiver wells to create a sink condition and minimize nonspecific binding. To reduce enzymatic degradation during the assay, protease inhibitors or cocktails [*e.g.*, aprotinin, 4-(2-aminoethyl) benzenesulfonyl fluoride (AEBSF), bestatin] are usually added to the system to reduce peptide proteolysis.

A number of *in silico* models have also been developed to predict permeability of peptides (13,35,36). Stenberg *et al.* found that using dynamic molecular surface properties, Caco-2 permeability of peptides was successfully predicted (35). Rezai *et al.* reported an atomistic physical model to predict PAMPA passive membrane permeability of cyclic peptides that did not involve "training data" (13). Rafi *et al.* discussed an all-atom force field-based method to calculate changes in free energy associated with the transfer of the peptidic molecules from water to membrane (36). The method correctly predicted rank order experimental permeability trends from MDR1-MDCK cells within congeneric series and was much more predictive than methods that do not consider three-dimensional peptide conformation (36). It was found that the intentional introduction of hydrogen bond acceptor–donor pairs in peptides can improve membrane permeability (36). The key descriptors for peptide permeability are hydrogen-bonding capacity (especially intramolecular hydrogen bonding), hydrophobicity/lipophilicity, size, and polar surface area (13,35,36). *In silico* models are particularly useful prior to peptide synthesis to estimate permeability values and when it is technically challenging to measure permeability experimentally due to stability, nonspecific binding, sensitivity, and other issues.

In vivo animal models are frequently used to study peptide bioavailability and fraction absorbed in nonsurgical or portal vein cannulated (PVC) animals. *In vitro* data can be used in conjunction with *in vivo* studies to determine whether low oral bioavailability is due to poor absorption or rapid first-pass liver extraction. Transporter knockout animals (*e.g.*, PEPT1 knockout mice) are useful to understand the contribution of uptake transporters in oral absorption (37). Bioanalysis of peptides can be challenging due to low sensitivity and selectivity, high nonspecific binding and protein binding, low recovery, carryover, solubility, and stability issues (38–40). For peptides with poor stability, blood/plasma samples need to be stabilized once they are removed from the *in vivo* systems (41). Protease inhibitors or cocktails (*e.g.*, aprotinin to avoid proteolysis or oxidation of cysthiols) are usually added to the collection tubes on wet ice to cool the samples immediately after blood collection and to prevent further hydrolysis during sample preparation and analysis (42). Nonabsorptive collection tubes are used to minimize nonspecific binding (http://www.labonline.com.au/products/48460-Eppendorf-LoBind-Tubes-and-Plates-and-epT-I-P-S-LoRetention-pipette-tips). Displacement proteins (*e.g.*, serum albumin) or peptides (structural analogs) are sometimes added to compete for the surface-binding sites (40). Organic solvents, acids, salts, or surfactants (*e.g.*, Triton X-100 or Tween-20) can be added to overcome nonspecific binding by increasing peptide solubility (40,42).

Strategies to Enhance Peptide Permeability

Many strategies have been developed to enhance peptide permeability, including *N*-methylation to reduce hydrogen bonding potential, cyclization to increase rigidity, and introducing intramolecular hydrogen bonds to reduce intermolecular hydrogen bonds and flexibility (30,36,43–46). Cyclosporine A, an 11-residue peptide, comprises all of these strategies in its structure, *i.e.*, cyclic backbone, seven *N*-methyl groups, and four intramolecular hydrogen bonds (5,47). Other approaches include stapled peptides (produced by connecting two amino acids to increase helicity, potency, stability, and permeability), prenylated peptides with farnesyl (C_{15}) and geranylgeranyl (C_{20}) chains, and pepducins (containing a short peptide derived from a GPCR intracellular loop tethered to a hydrophobic moiety) where the N-terminus of the peptide is lipidated with palmitoyl or other fatty acids (C_{12}–C_{18}) (48,49). Reversible lipidization (covalently attached long chain fatty acid to a peptide) has been shown to prolong plasma half-life and increase GI stability and oral bioavailability (50–52). It overcomes the limitations of a conventional lipidization approach (*e.g.*, incompatible reaction media, insoluble in water, low biological activity) (51).

Transporter-mediated processes play an important role in peptide oral absorption. In the study of a library of 54 cyclic peptides with different *N*-methylation patterns, it was found that the similarity of the backbone conformation with the well-absorbed peptides (*e.g.*, cyclosporine A and *N*-methylated somatostatin analog) was important for uptake by absorptive transporters in the GI lumen (30). SMVT has been shown to be a promising transporter to improve oral absorption of peptide–biotin conjugates (53). The strategy of using the vitamin B_{12} uptake system to deliver peptide orally has shown great potential (54,55). With the increasing knowledge and understanding of transporter–peptide structure relationships, it will increase the success of oral delivery of peptides.

Formulation can help improve oral absorption of peptides by using absorption enhancers. A large number of permeability enhancers have been reported to increase intestinal absorption of peptides, such as surfactants, bile salts, phospholipids, fatty acids, and glycerides (56–58). The

mechanisms by which the enhancers increase permeability are (20,21): (1) opening epithelial tight junctions reversibly to increase paracellular transport (*e.g.*, EDTA increases paracellular permeability by chelating the calcium that is required to form intercellular tight junctions) (59); (2) mildly perturbing the mucosal surface by altering membrane fluidity to enhance transcellular permeation (*e.g.*, transient permeability enhancers with medium chain fatty acids) (60); and (3) forming noncovalent complex with payload to be absorbed (*e.g.*, Eligen®) (61). Despite 50 years of research on oral permeability enhancers, clinical success has yet to be achieved (20). The major challenges of oral permeability enhancers are low and variable oral bioavailability and safety concerns. These hurdles are currently being addressed by both academia and industry (20). A number of peptide clinical trials with permeability enhancers are ongoing, and several of them have shown promising oral efficacy (20).

Proteolytic Stability

Peptides are susceptible to proteolysis by proteases or peptidases due to the amide bonds in their structures. Both luminally secreted enzymes (*e.g.*, pepsins, elastase, trypsin, and chymotrypsin) and brush border membrane-bound enzymes (*e.g.*, endopeptidases, aminopeptidases, and carboxypeptidase) play important roles in peptide proteolysis. More than 550 putative proteases are ubiquitously distributed throughout the body (62). Proteolysis is a major elimination pathway for most peptides, and clearance of peptides can exceed cardiac output due to blood degradation.

Structure–Proteolytic Stability Relationship

In general, the N-terminus residue of a peptide correlates to its half-life in plasma. Peptides with N-terminus containing Met, Ser, Ala, Thr, Val, or Gly typically have longer half-lives. Peptides with N-terminus containing Phe, Leu, Asp, Lys, or Arg usually have shorter half-lives. Peptide domains rich in Pro, Glu, Ser, and Thr are more prone to enzymatic degradation. Proteolytic enzymes, their substrates, and site specificities have been well documented (17,63). A number of software programs are available to predict peptide cleavage sites, such as PeptideCutter (http://web.expasy.org/peptide_cutter/), PROSPER (https://prosper.erc.monash.edu.au/), and CutDB (http://cutdb.burnham.org/login). In drug discovery, the proteolytic enzymes for a specific peptide are not always known. In practice, the sites of cleavage are typically identified using liquid chromatography–mass spectrometry (LC-MS/MS). Knowing the cleavage sites allows a well-directed modification of peptide structure to minimize enzymatic degradation (17). Hydrolytic products are sometimes tested for pharmacological activity as they might be active against the disease target.

Stability Assays

Peptides can be incubated with biological matrices to evaluate their stability (64–66). Both kinetic information (*in vitro* intrinsic clearance and half-life) and degradation products can be determined. The typical matrices of various species are as follows: (1) plasma/serum and blood to evaluate degradation in systemic circulation; (2) GI fluids [simulated gastric fluid (SGF), simulated intestinal fluid (SIF)], intestine brush border membrane vesicles (BBMV), and intestine microsomes or S9 to examine GI stability and predict oral bioavailability; (3) liver microsomes, S9, cytosol, and hepatocytes to study liver metabolism by the various liver enzymes; (4) kidney BBMV, microsomes, or homogenates to assess kidney degradation; (5) tissue homogenates to examine tissue stability; and (6) assay media and formulation vehicles to ensure acceptable stability. LC-MS/MS is used to monitor parent drug depletion and examine the structures of the degradation products (67,68). The information is used to guide structure modification to improve peptide stability.

Strategies to Stabilize Peptides from Proteolysis

Many approaches are available to enhance stability of peptides through structure modification (17,69). Some approaches not only improve stability, but also enhance other ADME properties, *e.g.*, cyclization can increase stability and permeability; conjugation to macromolecules can improve stability and reduce renal clearance (70–75). It is important to maintain potency and avoid toxicity while improving stability and ADME properties of peptides.

- Protecting N- and C-terminus

A number of proteolytic enzymes in blood/plasma, liver or kidney are exopeptidases, aminopeptidases and carboxypeptidases and they break down peptide sequences from the N- and C-termini. Modification of the N- or/and C-termini can often improve peptide stability. Many examples have reported that N-acetylation, and C-amidation increase resistance to proteolysis (8,76). For example, N-terminal acetylated somatostatin analogs were reported to be much more stable than the native peptide (69). The N-acetylated 7-34 form of glucagon-like peptide-1 (GLP-1 7-34) has been shown to be much more stable than the unprotected peptides (77). Even though N-acetylation and C-amidation are known to increase stability against exopeptidases, it was found to improve resistance against endopeptidases for EFK17 (EFKRIVQRIKDFLRNLV) peptide when applied in conjunction with amino acid substitutions (78). Tesamorelin has a hexenoyl group attached to the N-terminus tyrosine residue and has a much longer half-life (1 h) than the natural growth hormone-releasing hormone (GHRH, 6.8 min) (79).

- Replacing L-amino acids with D-amino acids

Substituting natural L-amino acids with nonnatural D-amino acids decreases the substrate recognition and binding affinity of proteolytic enzymes and increases stability. One example is vasopressin, which contains an L-Arg and has a half-life of 10–35 min in humans (80). The D-Arg analog, desmopressin, has a half-life of 3.7 h in healthy human volunteers (81). Octreotide, a drug for the treatment of gastrointestinal tumors, has a shorter amino acid sequence than the endogenous hormone somatostatin (8 vs. 14 amino acids) and differs by the substitution of L-amino acids with D-amino acids. The human in vivo half-life of octreotide improved to 1.5 h from a few minutes for

somatostatin (82). In the study of a bicyclic peptide inhibitor of the cancer-related protease urokinase-type plasminogen activator (uPA), replacement of a specific glycine with a D-serine not only improves potency by 1.8-fold but also increases stability by 4-fold in mouse plasma (83). In MUC2 epitope peptide, the partial D-amino acid-substituted peptide exhibited high resistance against proteolytic degradation in plasma and lysosomal preparation (84). However, exceptions do exist. Dermorphin analogs with additional D-amino acid substitutions were found to be more rapidly cleaved than the parent peptides, potentially due to remote secondary structural features that are important for enzyme recognition (85). In another case, D-amino acid-substituted analogs of growth hormone-releasing factor 1-29 amide did not show significant improvement of half-life in rats (86). These exceptions highlighted the importance of the understanding of key structural features and interactions to guide successful peptide modifications. Recently, the novel concept of D-peptide (minor image of natural all L-peptide) significantly improved stability and half-life. Rotigaptide (antiarrhythmic) and PIE12 (HIV) are two D-peptides in development (87,88). One major concern of using unnatural amino acids is the potential toxicity. Unnatural amino acid substitutions have been found to associate with adverse effects as they can accumulate in the liver and other organs (89,90).

- Modification of amino acids

Modification of natural amino acids can improve the stability of peptides by introducing steric hindrance or disrupting enzyme recognition (17). For example, gonadotropin-releasing hormone has a very short half-life (minutes), while buserelin, in which one Gly is replaced with a t-butyl-D-Ser and another Gly is substituted by ethylamide, has a much longer half-life in humans (http://products.sanofi.ca/en/suprefact-depot.pdf). Ipamorelin, a pentapeptide, has 2′-naphthylalanine and phenylalanine in the D configuration and the C-terminal L-alanine replaced by 2-aminoisobutyric acid, resulting in improved terminal half-life of ~2 h in humans (91,92).

- Cyclization

Cyclization introduces conformation constraint, reduces the flexibility of peptides, and increases stability and permeability. Depending on the functional groups, peptides can be cyclized head-to-tail, head/tail-to-side-chain, or side-chain-to-side-chain. Cyclization is commonly accomplished through lactamization, lactonization, and sulfide-based bridges (4). Cyclic enkephalin analog was found to be highly resistant to enzymatic degradation (93). A cyclic epitope peptide derived from herpes simplex virus glycoprotein was completely stable in 50% human serum, but the linear peptide was totally unstable (94). Stapled peptides have been shown to significantly enhance serum stability by reinforcing an α-helix to create a shield from proteolysis (95–97). ALRN-5281, a stapled peptide, is currently in a clinical trial for treating orphan endocrine disorders (98). Disulfide bridges create folding and conformational constraints that can improve potency, selectivity, and stability. A number of disulfide bond-rich peptides are on the market or in preclinical or clinical development, e.g., linaclotide, lepirudin, and ziconotide (15).

- Conjugation to Macromolecules

Conjugation to macromolecules (e.g., polyethylene glycol (PEG), albumin) is an effective strategy to improve stability of peptides and reduce renal clearance. This will be discussed in the "Renal Clearance" section.

Renal Clearance

Many peptides exhibit promising *in vitro* pharmacological activity but fail to demonstrate *in vivo* efficacy due to very short *in vivo* half-life (minutes). The rapid clearance and short half-life of peptides hamper their development into successful drugs. The main causes of rapid clearance of peptides from systemic circulation are enzymatic proteolysis or/and renal clearance. The glomeruli have a pore size of ~8 nm, and hydrophilic peptides with MW <2–25 kDa are susceptible to rapid filtration through the glomeruli of the kidney. Since peptides are not easily reabsorbed through the renal tubule, they frequently have high renal clearance and short half-life. Other minor routes of peptide clearance are endocytosis and degradation by proteasome and the liver. Comparison between systemic and renal clearance in animal models provides useful information on whether renal clearance is likely to be a major elimination pathway.

For renal-impaired patients, dose adjustment may be needed for peptide drugs to avoid accumulation and higher drug exposure, as inappropriate dosing in patients with renal dysfunction can cause toxicity or ineffective therapy (99,100). Several strategies have been developed to reduce peptide renal clearance and prolong half-life. These will be reviewed next.

- Increase plasma protein binding

Renal clearance of peptides is reduced when they are bound to membrane proteins or serum proteins. An example is the cyclic peptide drug octreotide, a treatment for endocrine tumors, which has about 100 min half-life in humans due to binding to lipoproteins (fraction unbound 0.65) (101,102).

- Covalent Linkage to Albumin-Binding Small Molecules

Covalently attaching albumin-binding small molecules to peptides can reduce glomerular filtration, improve proteolytic stability, and prolong half-life by indirectly interacting with albumin through the highly bound small molecules (74). Liraglutide is a GLP-1 analog that is linked via a γ-L-glutamyl spacer to a 16-carbon fatty acid residue (Fig. 2) (103). The lipopeptide binds to albumin, thus decreasing proteolysis and renal clearance (104–106). Half-life increased to 8 h after IV administration compared to a few minutes for native GLP-1. The SC half-life of liraglutide is 11–15 h, enabling QD dosing. Another example is a bicyclic peptide linked to an albumin-

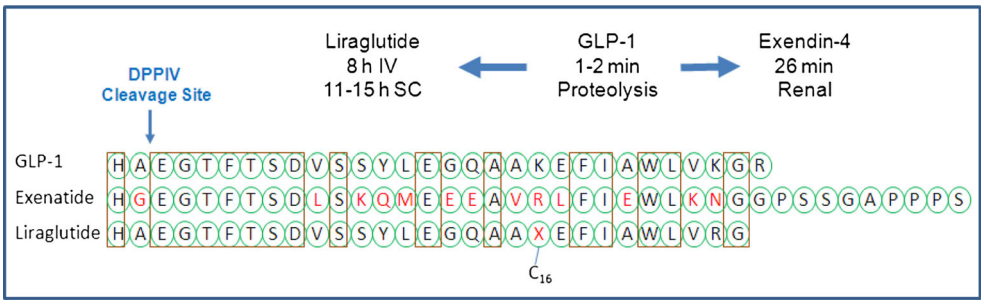

Fig. 2. Strategies to enhance peptide stability: GLP-1 peptide has a half-life of 1–2 min. Modification of labile amino acids resulted in exenatide having enhanced serum stability, and clearance shifted from proteolysis to renal clearance, half-life 26 min. Addition of palmitoyl chain (C16 fatty acid) to GLP-1 formed liraglutide with decreased proteolysis and renal clearance, half-life 8 h IV, 11–15 h SC enabled QD dosing

binding peptide (107). The conjugate was completely resistant to proteolysis and had a 50-fold longer half-life (107).

- Conjugation to Large Polymers

Conjugation of peptides to large synthetic or natural polymers or carbohydrates can increase their molecular weight and hydrodynamic volume, thus reducing their renal clearance. The common polymers used for peptide conjugation are PEG, polysialic acid (PSA), and hydroxyethyl starch (HES). An example is peginesatide, a PEGylated synthetic peptide approved by the FDA recently for the treatment of anemia associated with chronic kidney disease but was withdrawn as a result of new post-marketing reports of serious hypersensitivity reactions (http://www.takeda.com/news/2013/20130701_5854.html). It has an elimination half-life of 18.9 h after IV administration in healthy volunteers (108).

- Fusion to Long-Live Plasma Proteins

Plasma proteins, such as albumin and immunoglobulin (IgG) fragments, have long half-lives of 19–21 days in humans (74). Because of the high MW (67–150 kDa), these proteins have low renal clearance, and their binding to neonatal Fc receptor (FcRn) reduces the elimination through pinocytosis by the vascular epithelium. Covalent linkage of peptides to albumin or IgG fragments can reduce renal clearance and prolong half-life. An example is the albumin-exendin-4 conjugate (CJC-1134-PC). It has a half-life of ~8 days in humans and is currently in a phase II clinical trial for the treatment of type II diabetes mellitus (109). The recently FDA-approved drug, albiglutide, is a DPPIV-resistant GLP-1 dimer fused to human albumin and has a half-life of 6–7 days, which enables weekly dosing for the treatment of type 2 diabetics (110,111).

Predicting PK Parameters of Peptides

Predictions of peptide PK and PK/PD are important for candidate selection and dose regimen design. Peptide clearance mechanisms can be similar to either small molecules or proteins depending on their structures and physicochemical

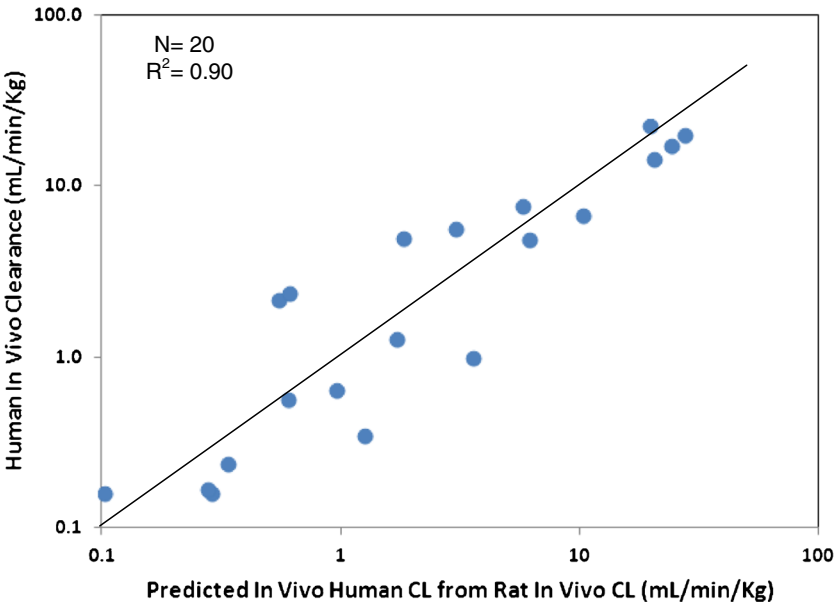

Fig. 3. Correlation of observed human clearance and predicted values from single species scaling from rat clearance for 20 peptides. CL_{human} (L/h)=CL_{rat} $(L/h)\times(BW_{human}/BW_{rat})^{0.75}$

properties. Certain small and lipophilic peptides (cyclosporine, bortezomib) have clearance mechanisms similar to small molecules (*e.g.*, *via* P450-mediated metabolism) (112–114). Strategies that apply to small molecule drugs can be used to scale human *in vivo* clearance of peptides (115). If peptides behave more like proteins that are eliminated through proteolysis, renal filtration, catabolism, and endocytosis, allometric scaling appears to be successful in predicting human PK parameters from preclinical species (22,116). A number of studies have shown that allometric scaling is effective in predicting human volume of distribution and clearance with some exceptions (22,117–120).

Because of the ubiquitous distribution of proteases throughout the body, proteolytic degradation is not limited to classical clearance organs (*e.g.*, liver, kidney). Allometric scaling has been shown to provide satisfying human clearance prediction in the absence of nonlinear PK and species-specific clearance mechanisms. In a comprehensive study of 34 therapeutic proteins, including 12 monoclonal antibodies and Fc fusion proteins, human clearance values were reasonably well predicted with simple allometric scaling and a fixed exponent of 0.8 (116). About 95% was within 2-fold of the observed values when using all available species (*e.g.*, mouse, rat, dog, monkey, rabbit) or about 90% using monkey single species scaling (116). Evaluation of single species scaling with a fixed exponent of 0.75 using rat clearance of 20 peptides showed good prediction of human clearance with a correlation coefficient of 0.90 (Fig. 3). Applying a fixed exponent with one to two preclinical species is simple, resource saving, and minimizes systematic bias compared to a fitted exponent method (116). However, due to potentially unrecognized pitfalls of allometric scaling (*e.g.*, species-dependent clearance mechanisms), special cautions (*e.g.*, understanding of clearance mechanisms) need to be applied when using this approach (22).

Peptides cannot cross biomembranes easily and, therefore, are mostly confined in the extracellular space. Diffusion and convection are both involved in the distribution of peptides and the relative contribution is dependent on the size and structure of the peptides (22). Volume of distribution of peptides is typically small and not greater than the volume of the extracellular body fluid (V_{ss}<15 L or 0.2 L/Kg). V_{ss} is reasonably well predicted with allometric scaling using animal data with an exponent near 1 (22).

Some peptides showed nonlinear PK caused by saturation of target-mediated drug disposition (TMDD). A combined model of TMDD and allometric scaling was able to simultaneously describe preclinical PK of exenatide from mouse, rat, and monkey following both IV and SC dosing (121). The model structure was successfully applied to predict human concentration–time profiles (121). The advantages of such mechanistic models compared to empirical models are their abilities to extrapolate from preclinical to clinical species and from healthy volunteers to disease state and special populations.

CONCLUSIONS

Development of peptide drugs is, no doubt, not only full of challenges and risks but also offers great potential and promise. Future enhancement of the ADME tools will help accelerate the development of peptides into successful drugs. Developing deeper understanding of physicochemical properties that govern peptide conformation is critical to assessing the impact on potency and ADME properties (*e.g.*, permeability, stability, and PK). Predictive *in silico* or rule-based ADME tools are useful to guide peptide design with improved drug-like properties while maintaining target potency. Transporters can play a vital role in uptake of peptides for enhanced oral absorption and cell membrane penetration. Effective and physiologically relevant transporter assays will help define substrate specificity and guide peptide design with improved uptake and transport characteristics. Continued refinement of mechanistic PK and PD models will provide powerful insights in designing future generations of peptide drugs with the greatest safety and efficacy.

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