Editorial

Theme: Preclinical Peptide Developability Assessment

Guest Editors: Annette Bak and Weiguo Dai

Peptide Developability at the Discovery-to-Development Interface—Current State and Future Opportunities

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Peptides represent an interesting and sparsely explored therapeutic niche between small molecule and large biologics. However, peptides, as a drug class, present a range of challenges that have restricted their therapeutic utility. Peptides are experiencing attrition in discovery and early development mainly due to lack of desirable physicochemical, formulation, drug metabolism, and pharmacokinetics properties, or unacceptable toxicity and lack of efficacy in preclinical and clinical studies.

To address these issues in R&D pipelines and prioritize the peptides with highest probability of success, we organized the 2013 AAPS symposium “Increasing Success for Peptides in Clinical Development—An integrated Developability Approach” and edited the AAPS Journal themed issue entitled “Preclinical Peptide Developability Assessment.” The articles published in this series (1–3) aim at providing guidance on conducting peptide developability assessment at the discovery-to-development interface, including how to use recent technological advances in peptide characterization and analytical tools for this purpose. The ultimate objective is to facilitate design and selection of lead candidates that can be advanced to the clinical stage.

“Early Engineering Approaches to Improve Peptide Developability and Manufacturability” by Furman et al. (1) discusses how the small size and greater flexibility of peptides, compared to large biologics, make them good drug candidates, particularly for cell surface and membrane spanning targets. In spite of the diverse properties of therapeutic peptides, scientists can now identify, through analysis of the primary sequence, the labile amino acids (e.g., Asp, Asn, Cys, Met, and Trp) as soft links in the peptides that can result in potential adverse post-translational modifications, which include deamidation,

isomerization, oxidation, and hydrolysis. Such changes often result in compromised biophysical and functional properties. As a result, assessment of the soft links in peptide sequences allows scientists to engineer the peptide early in the discovery stage by removing or replacing labile amino acids with those which are more stable. However, one must also consider the overall effect of peptide sequence changes on peptide developability. Therefore, engineering the sequence needs to be conducted iteratively to achieve optimized peptide properties (e.g., chemical stability) while preserving biological activity.

An additional liability is proteolytic degradation mediated by enzymes at epithelial barriers and in the circulation. Unmodified peptides generally undergo extensive hydrolysis via aminopeptidases (N-terminal cleavage), endopeptidases (cleavage sites away from terminals), and carboxypeptidases (C-terminal cleavage). Mitigation strategies include terminal modifications, incorporating modified or un-natural amino acids, cyclization, disulfide bond replacement, stapling to restrict conformation, or N- or C-terminal conjugation. If the chemical or enzymatic liabilities cannot be engineered out in the design phase, then it is critical to understand them thoroughly for a systematic development of the scale-up process either by chemical synthesis or recombinant expression. “Physicochemical and Formulation Developability Assessment for Therapeutic Peptide Delivery—A Primer” by Bak et al. (2) provides guidance and strategies on peptide developability assessment at the discovery-to-development interface for injectable, oral, and more briefly transdermal, intraoral, and respiratory peptide drug delivery. The parenteral route is the prevalent route of administration for peptides, and challenges include physiochemical properties (e.g., solubility and chemical liability) and biophysical stability (e.g., aggregation) along with adsorption to surfaces such as those of product containers. The risks associated with peptide molecular developability can be mitigated through enabling formulation technologies, which include buffer and pH screening, addition of antioxidants (against oxidation), surfactants and polymers (against adsorption), and salts (against denaturation).

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Significant efforts have been made in enabling oral delivery of peptides, as the route has increased patient preference and compliance over parenteral administration. Neoral, an oral product of cyclosporine A, was launched in 1994. The structural features of this cyclic peptide have made it relatively permeable and resistant to peptidase degradation, but it suffers from solubility-limited absorption. Cyclosporine A is a natural product, but the case study carries significant learnings for design scientists. It illustrates that strategies intended to increase permeability and reduce enzymatic degradation of peptides may lead to low solubility and hence a different set of absorption liabilities (see (2) for more details).

Characterization of orally delivered peptides includes not only assays classically used for orally delivered small molecules such as permeability assessment and food effect prediction, but also assays used for parenterally delivered peptides (i.e., physicochemical and biophysical properties and stability). Formulation approaches for orally delivered peptides are designed to achieve (1) solubilization (e.g., using surfactants, polymers), (2) protection against proteolysis (e.g., incorporation of protease inhibitors), and (3) increasing permeability (e.g., incorporation of mucoadhesive polymers and permeation enhancers).

Other alternative delivery routes including nasal, pulmonary, transdermal, and intraoral have been explored for peptide therapeutics. For example, inhaled insulin products have been approved (i.e., Afrezza®, Exubera®). These routes are commonly explored for enhancing patient compliance, convenience, and local use, but they can also be used for systemic delivery.

“Strategic Approaches to Optimizing Peptide ADME Properties” by Di (3) covers absorption, distribution, metabolism, and excretion (ADME) challenges encountered by peptide drug discovery scientists, and among these aspects, poor absorption and proteolytic liability of peptides usually present the major developability concerns. Assessing peptide absorption may employ parameters and assays such as a partition coefficient assay at pH 7.4 (e.g., Log D), parallel artificial membrane permeability assay (PAMPA), and in vitro permeability through Caco-2 cells, as well as in vitro proteolytic stability assays in various matrices such as plasma, GI fluids, liver, kidney, or other tissue microsomes or homogenates. Design strategies to increase absorption and proteolytic stability of peptides include modifying amino acids, cyclization, and chemical conjugation.

Another important topic to be addressed for peptides is human pharmacokinetic (PK) prediction. Clearance mechanisms for small lipophilic peptides may be similar to those for small molecules (e.g., cytochrome-P450-mediated metabolism) or those for proteins (e.g., proteolysis, renal filtration, or endocytosis). The PK method classically used for small molecules, allometric scaling, appears to be more successful when non-linear PK is absent. However, peptides can sometimes exhibit non-linear PK especially when target-mediated drug disposition is saturated, in which case the allometric method will have to be modified accordingly.

These articles therefore provide an overview of peptide developability assays for three preclinical functions at the discovery-to-development interface. One of the challenges facing scientists is how to select appropriate peptide developability assays when a large number of peptide candidates need to be evaluated with rapid turnaround times and limited material is available. The

process is therefore important, and the testing flow should be organized so that higher throughput and material sparing assays are conducted first, leading to a reduction in the number of candidates considered for more resource intensive assays. The drawback of such an assessment approach is that peptides passing the developability assessment may fail later in development due to limitations of the discovery assays.

In addition to the aspects presented in this series, the field of peptide developability can benefit from further insights around peptide efficacy, as nominating a candidate with a superior or at least adequate efficacy will be important for candidate success in clinical development. Those developability criteria will however be very dependent on therapeutic area. In addition, peptides have unique safety profiles, potentially encompassing element characteristic of small molecules (e.g., organ specific toxicity), and those of proteins (e.g., immunogenicity). Since there is, to the authors’ knowledge, no specific regulatory toxicology guidance for peptides, this topic could benefit from more illumination in the literature.

In spite of the approval of a number of peptide commercial products, there are currently limited guidance or publications concerning how to approach peptides in clinical development. In the formulation area, for example, researchers consult guidance documents and background information for small molecules or large biologics when making peptide product development decisions. As more peptides transition from discovery into clinical development, this area could benefit from further discussion in the scientific community.

Lastly, as evident from these articles, the majority of peptides are currently delivered by the parenteral route, although the oral route, while still applied only in a limited number of products, can present a significant advantage from a convenience/compliance perspective. Alternative routes of administration, such as transdermal, intraoral, intranasal, and inhaled delivery, may offer additional benefits. A key question is therefore, “How to select the most suitable administration route?” This question could be addressed by a decision tree that includes commercial and scientific aspects such as potency, biocompatibility/safety, and PK variability requirements specific to the target in question. Developability assessment for alternative delivery routes at the discovery-to-development interface will however be very challenging since drug performance is dependent on the formulation and delivery systems/devices, which are usually not available or selected until the late development stage.

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