Oral delivery of peptides: opportunities and issues for translation

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The intractable challenge of delivering oral peptides has exercised the minds of pharmaceutical scientists for decades, with cyclical periods of enthusiasm followed by depression. Moroz et al [1] recently cited the first failed attempt to achieve orally-delivered insulin in 1922 just one year after its discovery, so we are approaching a centenary of effort. The reasons for the initial failure of oral insulin emerged in the intervening years as comprising the many combined hurdles that a labile hydrophilic large molecular weight peptide must overcome, including attack by the acidic stomach pH and by the pancreatic and intestinal serine peptidases, as well as minimal intestinal epithelial permeability, and a large first pass effect for those peptides aimed at systemic delivery beyond the liver. A 1961 study to deliver another poorly permeable macromolecule (heparin) using EDTA as an epithelial permeation enhancer yielded positive data in dogs [2], but was not built upon, as there were toxicity concerns over the large doses of EDTA required. By the 1980s, studies using biocompatible nanoparticles containing insulin reduced blood glucose levels, especially in diabetic rats [3], and this data generated great excitement, however methodological differences, questions over dose levels, and issues around reproducibility and scale-up prevented translation for these and other nanoparticle constructs in the subsequent 25 years. Within the same time frame, simpler formulations using established excipients or molecules with a history of use in man emerged, and some of them have reached oral clinical trials for peptides including insulin, Glucagon-like Peptide 1 (GLP-1) agonists, salmon calcitonin and octreotide [4]. For most of these agents however, even the most promising current formulations, based either on permeation enhancers, peptidase inhibitors, or nanoparticle constructs, do not appear to be yielding oral peptide absolute bioavailability values of >1-2% in human trials.

Despite that, we are in a phase of anticipation with renewed interest in oral peptides by the majority of large Pharma companies as well as from funding agencies. What is the basis of this excitement? Firstly, synthetic and recombinant peptides are manufactured more cost-effectively than ever and therefore relatively low oral bioavailability values may still be commercially acceptable for potent peptides with high therapeutic indices. Secondly, industry is making more stable low molecular weight and less hydrophilic cyclic peptides [5] and lipidic peptide pro-drugs [6] with a real potential for oral delivery. On this point, it is instructive that the two marketed oral peptides, cyclopsorine A and desmopressin, as well as octreotide in an oral formulation (completed a Phase III trial) each have macrocycle conformations. Moreover, there is realization that many molecules including peptides may still have considerable oral bioavailability despite not conforming rigidly to the Lipinski rules [7]. Thirdly, the rationale for oral delivery is a sophisticated argument beyond that of just compliance and convenience. For example, the liver target of insulin and GLP-1 agonists can be accessed more physiologically and in much higher concentrations via the hepatic portal vein than from subcutaneous delivery, thereby reducing systemic exposure with its attendant side-effects. In addition, oral delivery options for insulin and GLP-1 analogues would assist moving Type 2 diabetics onto these molecules earlier in the disease to generate better clinical outcomes. In the example of oral octreotide, the convenience argument is much more obvious than for other types of peptide injections, as patients with acromegaly have to endure the misery of weekly octreotide administration with large gauge needles [8], a very different scenario to the highly-advanced pen-injectors for insulin and GLP-1 agonists. Fourthly, new technologies and formulation approaches as well as new physicochemical characterization techniques to examine the nano-constructs have significantly advanced over the last decade i.e., we can have more confidence concerning the role of each component in a formulation so that prototypes can emerge in a more systematic fashion. Fifthly, advances in imaging and better in vitro and in vivo bioassays provide crucial information for the understanding of the mechanism of action of constructs and this may lead to a more rational design. Finally, there are more oral peptide formulations in advanced clinical trials than ever before and this provides encouragement that a translational and regulatory path is available.

In 2012, the European Union’s FP7 programme funded the TRANS-INT consortium of seventeen partners from academia and industry to re-investigate how nanoparticle constructs could be used to deliver oral peptides [9]. The Coordinator (MJA) and Deputy Coordinator (DJB) approached ADDR in 2015 with a proposal to harness the considerable talent across the wide range of TRANS-INT partners, scientific advisors, and associated researchers to put together a Theme Issue with a particular focus on translation of oral peptide delivery technologies, mostly those based on nanoparticle technology. The philosophy behind the Theme Issue was to break it down into several areas: the current clinical trial landscape for oral peptides, the potential development path for a nanoparticle oral peptide construct, and the importance of human cell culture and intestinal tissue bioassays in screening nanoparticles. It continues with reviews on a range of targeted and untargeted nanoparticle classes, how cell penetrating peptides and intestinal permeation enhancers can be incorporated in formulations, and the importance of establishing PK/PD relationship for peptide-loaded nanoparticle formulations. It concludes with reviews on the issues of whether there are truly predictive in vitro toxicity assays for oral peptide nanoparticles, as well as the relevance of local intestinal immunological toxicity assays and hypersensitivity screens for particle prototypes.

Introducing the Reviews in detail, Lakkireddy et al [10] from Sanofi Pharma examine the challenges involved in translation of nanoparticle-
based approaches for anti-diabetic peptides and provide a bespoke development plan. They emphasize the importance of in vitro characterization of prototypes using modern tools, as well as that of the latest in vivo imaging technologies intended to provide information on the behavior of the nanoparticle at the human intestinal epithelium in vivo. By gaining better understanding of the mechanism of action in vivo, studies can be appropriately designed for relevant animal models. Aguirre et al [4] provide what we believe to be the most extensive analysis of the current clinical trial data of oral peptide prototypes in the literature to date. This data is hard to access, as most of it is not in the peer-reviewed literature, but this academic-industry team used an extensive range of resources in an effort to provide a balanced analysis. With the rapidly changing landscape of clinical development of oral peptides, the “current status” designation in the title will surely be already out of date. Their somewhat tempered conclusion is that even the most advanced formulations are still only providing 1-2% oral bioavailability and that the current formulations in trials are only offering incremental gains. In highlighting preclinical advances, they also alert readers to the “high risk-high gain” potential for step changes in oral bioavailability using drug-device combinations based on microneedles and intestinal patches.

Continuing the theme of using improved bioassays to screen and compare nanoparticle formulations in vitro, Beloei et al [11] focus on the use of in vitro human intestinal epithelial monolayers, mucous-secreting co-cultures, cytokine-activated monolayers, and M-like cell co-cultures to assess the mechanism of polymeric and lipid-based particle uptake, along with attempts to rank order the degree of uptake in relation to particle diameter, composition, surface charge and the presence of targeting ligands. They predict that 3D intestinal models are likely to become important in screening particle prototypes and will be complementary to the Transwell®-based systems that have been central for almost three decades. Lundquist and Artursson [12] review the use of isolated human intestinal tissue mucosae under a range of electrophysiological and toxicological criteria in Ussing chambers and compared data with tissue culture models under a set of criteria. They show that advanced microscopy techniques can track fluorescent dual labelled nanoparticles with peptide payloads in tissue and can assess which types are likely to have a higher proportion internalized or adhered to overlying mucous. Advanced analysis of the proteome of intestinal tissue was provided in respect of improving understanding of the role of transporters, carriers and metabolizing enzymes in the small intestinal epithelium and as to how this impacts peptide transport. An especially attractive aspect of this Review was the attempt to relate cartoons of intestinal enteroocyte structure and associated receptor/carrier expression with true immune-stained histological images from the Human Protein Atlas [13].

No issue on oral peptide formulations could be complete without an analysis of the field of intestinal permeation enhancers. This is highly relevant because such agents can be considered as components of nanoparticle formulations, either co-entrapped or as part of the overall formulation. Maher et al [14] provide an extensive study of how candidate enhancers have evolved, their preclinical and clinical stages of progress, and some of the toxicity considerations. Extensive reference Tables are provided to compare them in terms of peptides candidates, stage of development, and mechanism of action. Importantly, they attempt to tidy up the literature by updating reviewers on which agents have long been discarded (but which still get mentioned in some reviews as still being in development), as well as new ones in preclinical stages. Malhaisse et al [15] discuss the physiological barriers that a nanoparticle containing a peptide must overcome in the GI tract. Detail is provided on how the particle surface may be modified by protein in the intestinal milieu, how particles may traverse or remain adherent to intestinal mucous, and how particles of various composition and shape may cross the epithelium by clathrin-, or caveolae-mediated endocytosis, or by micropinocytosis.

The next set of articles focus on particular classes of nanoparticles specifically in relation to oral peptides. Niu et al [16] review lipid-based nanoparticle systems for oral peptides. They discuss lipid selection for a range of constructs including solid lipid nanoparticles, liposomes, nanocapsules, self-emulsifying delivery systems, and emulsions. They predict that lipid-based excipients will be important components of multifunctional nanoparticle systems since they can combine a range of mechanisms including protease inhibition and permeation enhancement; such particles can be formulated to be highly loaded with peptides and to display a controlled release profile. Sanchez-Navarro et al. [17] then continue the theme of examining additional agents that may be considered as coatings of nanoparticles or as internal components. In their case, they focus on cell penetrating peptides (CPPs) including poly-arginine-based systems (PenetraMax™, penetratin, and mellitin). They review data on oral insulin in rats using conjugated- or electrostatically-attracted CPP mixtures, and one of their conclusions is that CPPs formulated with nanoparticles may provide an impetus for epithelial internalisation if the mucous barrier can be overcome. Griffin et al. [18] explore the issues surrounding physiological-based pharmacokinetic analysis of oral peptide nanoparticle-based systems. In a forward-looking piece, they discuss how nanoparticle constructs can alter the ADME of the peptide and that mechanistic and imaging-based studies will be needed to relate such changes to safety and efficacy, with considerable implications for regulatory assessment.

The final two reviews relate to unique toxicological issues for oral nanoparticles. Caipellano et al. [19] discuss in vitro assays for nanoparticle toxicological assessment. In the absence of a common testing strategy, they discuss whether common assays used for conventional pharmaceutics are relevant for oral nanoparticles? The authors illustrate potential mechanisms of nanoparticle toxicity, advocate the use of agreed standard operating procedures of assessment, and attempt to relate concentrations of nanoparticles and duration of exposure in relevant cell types to the in vivo situation. Finally Orfi and Sjebeni [20] advance this topic by analyzing the potential for oral peptide nanoparticle formulations containing excipients to induce local intestinal mucosal immune responses through their internalization by intestinal dendritic cells and macrophages. They also review the potential for such carrier systems to induce hypersensitivity and pseudo-allergy through complement activation and also via histamine-mediated mechanisms, and consequently advocate early testing in preclinical murine and large animal models of local immunity and hypersensitivity, as well as investigation of microbiome changes in the intestinal lumen. Although the direct evidence for such risk from oral nanoparticle formulations is limited to date, this somewhat controversial article should stimulate debate and raise awareness amongst researchers.

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References