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Review

Oral Controlled Release Technology for Peptides: Status and Future Prospects

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In spite of significant efforts in academic and commercial laboratories, major breakthroughs in oral peptide and protein formulation have not been achieved. The major barriers to developing oral formulations for peptides and proteins include poor intrinsic permeability, lumenal and cellular enzymatic degradation, rapid clearance, and chemical and conformational stability. Pharmaceutical approaches to address these barriers, which have been successful with traditional, small, organic drug molecules, have not readily translated into effective peptide and protein formulations. The success achieved by Sandoz with cyclosporin formulations remains one clear example of what can be achieved, although it is likely that effective oral formulations for peptides and proteins will remain highly compound specific. Although the challenges are significant, the potential therapeutic benefit remains high, particularly with the increasing identification of potential peptide and protein drug candidates emerging from the biotechnology arena. Successful formulations will most likely require a systematic and careful merger of formulation and design delivery systems which maximize the potential for absorption across the epithelial cell layer.

KEY WORDS: oral drug delivery; peptide absorption; absorption enhancers; enzymatic degradation; chemical stability.

INTRODUCTION

Major efforts, in both academic and industrial laboratories, have been directed toward developing effective oral formulations for peptide and protein drug candidates for the past several decades. The increasing number of peptide and protein drugs which are being identified in the biotechnology industry will only serve to accent the importance of such development activities. However, in spite of these major efforts, relatively little progress has been made in reaching the target of safe and effective oral formulations for peptides and proteins.

The difficulties associated with developing effective oral formulations for peptides and proteins have been elucidated in a number of excellent review articles (1–5) and will not be repeated in an exhaustive manner here. However, the main barriers to success are normally ascribed to: i) poor intrinsic permeability of peptides and proteins across biological membranes due to their hydrophilic nature and large molecular size; ii) susceptibility to enzymatic attack by intestinal proteases and peptidases; iii) rapid post-absorptive clearance; and iv) chemical instability, including tendencies to aggregate and/or nonspecifically adsorbed to a variety of physical and biological surfaces. Although the development of many traditional drug candidates also encounter similar barriers to success, peptides and proteins seem to be highly susceptible to all of these factors and the options available to the pharmaceutical researcher are more limited when dealing with peptides and proteins. For example, in development of traditional small, organic drug molecules, synthetic chemistry approaches are often successful in ameliorating one or more of the barriers to efficacious *in vivo* absorption (e.g. analogs may be designed which confer improved partitioning behavior or diffusivity without compromising biologic activity). Due to the much more complex chemistry (both chemical and conformational) of peptides and proteins, rational approaches to changing physicochemical properties without altering biologic activity are often not as readily apparent. And although some successes have been achieved, especially with smaller peptides, the likelihood for success with larger peptides and proteins remains relatively limited. To be successful, peptide and protein formulations will have to simultaneously address all of the issues which result in poor bioavailability: intrinsic permeability, degradation, rapid clearance, and chemical and conformational stability.

CURRENT STATUS OF ORAL PEPTIDE ABSORPTION

Permeability and Absorption Enhancers

The poor intrinsic permeability of peptides and proteins across biological membranes is well documented (6) and can generally be attributed to their hydrophilic nature and large molecular size. Membrane carrier systems which facilitate the absorption of small peptides (di- and tri-peptides) are not effi-

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cient at transporting larger peptides (7). Permeation enhancers have received considerable attention in attempts to modify the basic barrier properties of the intestinal epithelial cell membrane. A variety of enhancers, including salicylates (8), mixed bile salt-fatty acid micelles (9), chelators (10), fatty acids (11), acylcarnitines (12), surfactants (13), and medium chain glycerides (14) have been shown in cellular and animal models to increase the absorption of a variety of peptides. In all cases, bioavailability was still fairly low and variable. The extent of bioavailability which must be achieved to develop useful oral controlled release dosage forms is largely peptide specific. Where manufacturing cost is low and the peptide has a large therapeutic index, relatively low bioavailability (e.g. <10%) may be acceptable. For peptides where manufacturing costs are high and/or there is a narrow therapeutic index, significantly higher bioavailability (e.g. 30–50%) may be required. For those peptides which require high oral bioavailability in order to develop a safe and commercially feasible product, the challenges facing the pharmaceutical scientist will be significant. Even to achieve some relatively modest degree of bioavailability with most peptides, some perturbation of the biological membrane is necessary. Recently, Bjork, et al., (15) demonstrated that degradable starch microspheres applied to the apical surface of Caco-2 cell monolayers reversibly increased insulin flux, although the effect was 20-fold lower than that seen with mannitol as the model compound. Histologic evaluation indicated an opening of paracellular spaces, possibly by development of paracellular hydrostatic pressures during hydration of the starch microspheres. Scott-Moncrieff, et al., (9) reported increased insulin absorption following direct jejunal administration in dogs of a 30 mM sodium glycocholate and 40 mM linoleic acid mixed micelle formulation, although the apparent bioavailability was still only 1.8%. This same formulation approach elicited 41% insulin bioavailability in a rat loop model and the authors proposed that the much reduced effect in dogs was possibly due to dilution and spreading of the formulation resulting in a reduced concentration of insulin at the barrier membrane and increased exposure of insulin to proteolytic enzymes. Similar results have been obtained in our laboratories (16) and support the observation that localization of formulation components at a critical concentration on the cell layer is essential for activity. Mesiha and Sidhom (17) achieved significant glucose lowering activity in rabbits when insulin was administered with sodium salicylate in a medium-viscosity hydroxypropylcellulose vehicle to improve localization of the formulation at the administration site. Hosny, et. al., (18) also demonstrated salicylate activity in a rat model employing surgically placed enteric-coated tablets containing 20 mg sodium salicylate. Although a bioavailability of approximately 13–14% was reported, the pharmacologic activity of salicylate at this dose (scaled to human dosage forms) probably precludes its use as an enhancing agent for insulin. Co-administration of 20 mM D-glucose or D-xylose facilitated the transcellular flux of octreotide, an octapeptide, across Caco-2 monolayers, possibly by activation of the Na^+-dependent glucose transport with resultant increases in water flux (the effect was inhibited by phlorizin) (19). The effect observed *in vitro* was, however, only approximately 2-fold and one would expect a lesser effect *in vivo* where localization of the formulation at the cell barrier cannot be achieved as intimately as it can be with an *in vitro* cell monolayer model. Acylcarnitines (12) have also been shown to specifically increase paracellular spaces in epithelial cell layers and some limited data suggest improved flux of peptides (e.g. insulin) across cell layers in response to these enhancers. However, the dimensions of the paracellular space, even when "loosened" by formulation excipients, are still relatively narrow (<30–40 angstroms) and will not likely allow adequate aqueous diffusion of larger molecules like polypeptides and proteins.

Protection from Enzymatic Degradation

The susceptibility of peptides and proteins to enzymatic attack is well known and remains a major challenge of formulation efforts (7,20). Chemical modification of small peptides has been successful in protecting certain peptide structures from enzymatic attack without significant loss of biologic activity (21). Less success has been achieved with larger polypeptides due to the more complex nature of these compounds and this approach will not be reviewed here. Rather, attempts to protect peptides from enzymatic attack will be the focus of this discussion. Yamamoto, et al., (22) reported that various protease inhibitors, including sodium glycocholate, camostat mesilate and bacitracin can increase the glucose-lowering activity of insulin administered to rat small intestine and colon, apparently by inhibiting protease activity in the lumen and mucous layer of the intestinal tissue. Others have reported similar results (23,24) indicating the potential utility of protease inhibitors for improving intestinal peptide absorption.

Another approach to providing protection against proteolytic attack, rather than enzyme inhibition, has been to protect peptides or proteins in the physical environment of the formulation itself. In recent years, significant efforts have been directed toward formulating peptides in microemulsions (5), small particles, e.g. nanoparticles (25), and bioadhesive particles (4). The rationale in all three cases is often similar: protection of peptides from the intestinal environment prior to absorption and localization of the peptide at or near the cellular membrane to optimize the driving force for passive permeation. While some success has been achieved in animal models (26), this technology has not yet translated into effective oral peptide formulations for human use.

Rapid Clearance

As mentioned in the previous section, many peptide and proteins are susceptible to presystemic metabolism and this is not limited to hepatic extraction. Significant intestinal epithelial cell enzymatic activity is the first post-absorptive barrier to achieving therapeutic systemic peptide levels. Unlike many traditional drug candidates, peptides are also highly susceptible to enzymatic degradation in the circulating blood (32). Since this review is focusing on formulation issues, the area of post-absorptive metabolism and rapid peptide clearance will not be further discussed. But researchers must be aware that achieving significant absorption will not necessarily result in significant bioavailability due to the several layers of metabolic barriers which exist for peptides.

Chemical Stability and Aggregation

Peptides and proteins often possess physical properties which present significant formulation problems not encountered with many small, organic drug molecules. Because of the com-

plex nature of peptides, self-aggregation is always a concern in formulation efforts. The tendency of insulin to form hexamers is well documented and the absorption of hexamers will most likely be very different than monomer absorption. Hovgaard, et al., (33) reported the use of alkyl saccharide surfactants (e.g. dodecyl maltoside) to minimize insulin aggregation. The insulin-dodecyl maltoside complex also afforded some protection against enzymatic degradation. Human calcitonin is also known to self-organize into fibrillar structures with reduced biologic activity (3). Larger aggregates will also, most likely, result in poorer membrane permeability. The use of various surfactant approaches to maximize monomer concentration during peptide release may afford advantages in minimizing the size of the complex which must cross epithelial cell layers.

PEPTIDE DRUGS AS CANDIDATES FOR ORAL CONTROLLED RELEASE SYSTEMS

Although only very limited success (e.g. cyclosporin) has been achieved in developing and marketing oral peptide systems, interest remains extremely high. Major efforts, particu larly in the biotechnology arena, in identifying and manufacturing peptidic drug candidates has provided a renewed challenge for the pharmaceutical scientist to develop safe and effective oral peptide formulations as alternatives to first-line parenterals.

Many peptide drug candidates, some of which are currently available as parenterals, have undergone significant oral formulation efforts. The availability of safe and effective oral formulations for insulin, growth hormone, calcitonin, vasopressin, somatostatin, growth factors, leuprolide, interferon and other peptide or hormonal agents would provide a major advancement in treating a variety of diseases which currently require repeated parenteral administrations. In addition to these more traditional hormonal drug candidates, drug discovery efforts in the biotechnology industry will continue to identify peptidic compounds with significant therapeutic targets suitable for oral administration if effective systems can be developed. The advantages of oral systems in terms of patient compliance and acceptability is further augmented by the potential cost savings to the health care industry which could result since oral formulations do not require sterile manufacturing and administration can be effected without direct involvement of the health care provider. Although the challenges to developing oral peptide formulations remain significant, the potential advantages of breakthrough technology in this area justify continued efforts to identify and optimize both pharmaceutical and biological approaches for maximizing peptide absorption.

CHALLENGES REMAINING IN THE DEVELOPMENT OF ORAL CONTROLLED RELEASE PEPTIDE SYSTEMS

The future utility of permeation enhancers for peptide and protein delivery, as for many small organic drug molecules, will be a balance of effectiveness and safety. As mentioned previously, peptides and proteins only very poorly cross the intact epithelial cell layer of the gastrointestinal tract because of their size and hydrophilic nature. To achieve therapeutic systemic levels, some degree of compromise on the integrity of the cell layer will undoubtedly have to be achieved. The key for success, in terms of safety, will be the specificity of the permeation enhancement and the reversibility which can be achieved on chronic dosing. This is an area which has not been fully explored and remains a critical roadblock in the development of permeation enhancers for peptides and proteins. The efficacy of various permeation enhancers will also depend on the ability to co-deliver the peptide and enhancer at effective concentrations in a fashion which localizes the formulation at the epithelial barrier membrane. Most animal studies which have shown effective peptide delivery have involved some sort of physical restriction (e.g. ligated loop models) of the formulation at the desired site. To achieve this same sort of localization via an oral delivery system will be an additional key to successful use of permeation enhancers.

If formulations can be developed which incorporate effective protease inhibitors, this can afford a significant advantage in terms of effecting therapeutic peptide absorption. Two other factors are important, however, in terms of inhibiting proteolytic activity. The processes involved in peptide absorption (i.e. delivery or peptide release, absorption, proteolytic attack) are all interrelated and depend on the kinetics of each individual process. It is not clear whether simultaneous release of protease inhibitors along with a given peptide is the optimal pattern. It may well be that "programmed-release" of inhibitors prior to peptide release is necessary to inhibit enzyme activity sufficiently to maximize peptide absorption. How this can be achieved in the dynamic environment of the gastrointestinal tract is not clear at this time.

Ultimate success with protease inhibitors may rely on administration with controlled-release dosage forms designed to release peptide and inhibitor in the distal portions of the intestinal tract. Several studies have indicated reduced enzyme activity in distal intestine (7) which may facilitate the efficacy of protease inhibitor formulations if delivery can be reproducibly targeted to this area. An extension of this idea is colon targeting which has received significant attention in a number of laboratories (27–29). The rationale behind colon delivery is to avoid the enzymatic activity present in the small intestine. Colon delivery, even if advantages are gained in reduced enzymatic activity, is not without its own pharmaceutical issues, including the effects bacterial activity, interference from fecal material, longer disintegration and dissolution times in the colon milieu, and the ability to reproducibly target drug release to the colon. Effective colon delivery systems have not yet been developed and their future success will depend on the ability of researchers to overcome the additional problems unique to the colon environment.

Protection against lumenal enzyme attack is only one aspect of the problem. Bai and Chang (30) recently reported the presence of insulin-degrading enzyme (EC 3.4.22.11) in rat intestinal enterocytes. Taki, et al., (31) showed extensive metabolism of metkephamid before and during absorption across rat intestinal tissue indicating both lumenal and cellular enzymatic degradation. Therefore, even if lumenal enzyme activity is blocked, intracellular enzymatic degradation may still present a formidable barrier to successful transport of certain peptides through the cytosolic compartments of intestinal cells. If many peptides and proteins are too large to pass through paracellular spaces, cytosolic enzyme barriers may present a more difficult obstacle to overcome than lumenal enzyme degradation.

The technical difficulties associated with peptide or protein oral delivery remain challenging. While significant progress has been achieved with each of the obstacles individually and with certain peptides (e.g. inhibition of insulin degradation via prote-

ase inhibitors), the development of a composite formulation which improves permeability, protects against enzymatic degradation, overcomes rapid metabolic clearance, and satisfies chemical instability and aggregation concerns has not yet been achieved. Given the results which have been achieved in animal models, it is probably unrealistic to expect formulations to be developed for most peptides which can achieve high levels of absorption (e.g. >25%) from the gastrointestinal tract. The cyclosporin formulations developed by Sandoz remain the one clear example of a peptide which has been effectively formulated for oral delivery and which achieves a reasonably high level of bioavailability (>30%). Since cyclosporin is metabolically stable, one less barrier had to be overcome in its development. Significant obstacles with other peptides must be overcome before we can determine whether the cyclosporin success is unique to this peptide or whether significant success can be achieved with other peptides. It is clear that a well-controlled, rational formulation design process is necessary. The problems with peptide and protein delivery are not trivial and will not be overcome by trivial solutions. The opportunity for success remains, but it will most likely encompass careful formulation efforts with selection of appropriate peptide candidates and designed delivery systems. Since the barriers to peptide and protein absorption (permeability, enzymatic degradation, metabolism, chemical stability and aggregation tendency) will likely exhibit significant peptide specificity, formulations will have to be developed and tested for efficacy and safety on a case-by-case basis. It is doubtful that generic formulations will be identified which will be generally applicable to a variety of peptides. Although basic concepts may apply, significant peptide-specific formulation efforts will be required.

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