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PEPTIDE THERAPEUTICS

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1.1 HISTORY OF PEPTIDES AS DRUGS

The advent of molecular biology and our understanding of the physiological and pathological functions of peptides, coupled with advances in synthetic methodologies and peptidomimetics, marked the beginning of a new era in peptide and protein therapeutics, with the vision that there should be no limit to what can be produced as therapeutics. During that period a number of great peptide drugs such as Sandostatin, Lupron, Copaxone, and Zoladex were developed with great therapeutic benefit. The number of approved peptide drugs, however, remains low.

It was not until the last decade that we have seen a significant surge in the number of peptide therapeutics on the market (Figure 1.1). While 10 peptides were approved between 2001 and 2010, the current decade has thus far witnessed the approval of six new peptide therapeutics – a remarkable yearly increase [1, 2]. The number of peptides in development is also steadily growing roughly doubling every decade (Figures 1.2 and 1.3), and there are 400–600 peptides in preclinical studies. This is due to the advances made in our understanding of peptide stability, peptide synthesis, and formulation over the last three decades. Although the market share of peptide drugs is still relatively small (about 2% of the global market for all drugs), the approval rate for peptide drugs is twice as fast as the rate for small molecules, and the market is growing similarly at a rate that is twice the global drug market [3, 4].

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While encouraging, the potential for peptide therapeutics is far greater than what it is today.

1.2 FACTORS LIMITING THE USE OF PEPTIDES IN THE CLINIC

A number of factors have thus far limited the explosion that needs to happen in the peptide field. With the exception of a few peptides, the approved drugs so far target the extracellular compartment, and thus have to compete with biologics. Of the

<table>
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<th>Trade name</th>
<th>Generic name</th>
<th>Target</th>
<th>Indication</th>
<th>Year</th>
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<td>Forteo</td>
<td>Teriparatide</td>
<td>PTH1R agonist</td>
<td>Osteoarthritis</td>
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<td>Protein–protein inh.</td>
<td>HIV</td>
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<td>Ca(^{2+}) channel inh.</td>
<td>Pain</td>
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<td>Exenatide</td>
<td>GLP-1 R agonist</td>
<td>T2 diabetes</td>
<td>2005</td>
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<td>Symtin</td>
<td>Pramlintide</td>
<td>Calcitonin agonist</td>
<td>T1/T2 diabetes</td>
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<td>Eritropoietin analog.</td>
<td>Anemia</td>
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<td>Teduglutide</td>
<td>Guanidyl peptide analog</td>
<td>SBS</td>
<td>2012</td>
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**Figure 1.1** Peptide therapeutics marketed since 2002. (See insert for color representation of this figure.)

**Figure 1.2** Peptides in development over the last three decades. (See insert for color representation of this figure.)

While encouraging, the potential for peptide therapeutics is far greater than what it is today.
extracellular targets, GPCRs represent the major class, and in most cases, the peptides are agonist. GLP-1 represents one-third of these GPCR targets. We have seen a great advance in extending the circulating half-life of the peptides through the use of unnatural amino acids and formulation technologies, but have not yet reached the half-life achieved by antibodies. The delivery of peptides is still in the great majority of cases limited to i.v. (intravenous), s.c. (subcutaneous), or intranasal. Finally, safety is still a concern as better tissue selectivity is required.

To dramatically heighten their impact, peptides need to access the intracellular space to target protein–protein interactions. These interactions represent a vast source of potential targets with significant biological impact (there are estimated 300,000 such interactions in the cell), and will not in the majority of cases be modulated by small molecules. Peptides and biologics, given their relative size and ability to bind to extended surface areas, are the perfect candidates to inhibit protein–protein interactions. The duration of action of peptides needs to be extended, and while peptides are inherently selective against their targets, they need to more selectively distribute to the desired tissue. Finally, the route of administration needs to be expanded to include oral delivery.

1.3 ADVANCES THAT HAVE STIMULATED THE USE OF PEPTIDES AS DRUGS

The many great technological advances that started over a decade ago in drug delivery, peptide design, and synthesis are now maturing, and will undoubtedly address these key challenges and revolutionize the field over the next decades. Many of the technological advances are already proving that it is possible to make peptides permeable to cells, target tissues, have longer half-lives, and be orally bioavailable.

The discovery that certain peptides can penetrate cells and can, therefore, be an effective therapeutic on their own or alternatively bring other drugs into cells allowed for the first time to imagine targeting the intracellular compartment (Figures 1.4
HIV-enveloped protein tat was one of the first to be recognized for its cell-penetrating ability and, therefore, its potential use to carry bioactive cargo into the cell [6]. Since 2004, more than 200 peptides carried into cells by tat or other naturally occurring cell-penetrating peptides (CPPs) have been in various phases of development [7]. However, the more recent advances in the understanding of how these peptides cross the cell membrane through endocytosis and/or macropinocytosis [8] has allowed the generation of CPPs with intrinsic biological activity [9–12]. It is now possible to take a CPP sequence and synthetically modify it to introduce the key amino acids of an effector peptide into its sequence and create potent peptide antagonists of an intracellular protein–protein interaction with good pharmacokinetic properties [13].

1.4 DEVELOPMENT OF PEPTIDE LIBRARIES

By looking at the list of CPPs in development, one realizes that they are single cases and have to be synthetically prepared and modified to impart some of the desired stability to be a useful therapeutic. It is hard to compete with the screening of the millions of small molecule compounds in various pharmaceutical companies and more recently in many academic centers.

Until now, the available technologies to screen large libraries of peptides of significant length (possessing secondary structure) would only allow us to generate large libraries of natural amino acid sequences through phage display, and if unnatural
DEVELOPMENT OF PEPTIDE LIBRARIES

Figure 1.5 Orally stable and bioavailable peptides (a) Cyclosporin. (b) Destruxin. (c) Kalata B.

amino acids were to be introduced, it had to be done with conventional synthetic methodology, and thus be limited to very low numbers of peptides that can be prepared and screened.

Indeed, over the last decade, there has been an explosion of very elegant technologies that now allow the generation of large to extremely large libraries of linear and macrocyclic peptides with unnatural amino acids and unnatural linkers. For the first time, it is possible to engineer stability, cell permeability, and possibly oral bioavailability at once and screen for the desired properties very rapidly. These major advancements have resulted in the generation of a number of companies that are pushing the limits of these technologies to rapidly screen and identify novel peptide therapeutics against protein–protein interaction targets (Figure 1.5).

Ensemble therapeutics utilizing their DNA-programmed chemistry can generate million-member libraries of small macrocycles with MW of 500–1500. On screening these libraries, they have identified potent and orally bioavailable small molecule inhibitors of IL17 [14]. Through medicinal chemistry optimization, they have now identified picomolar inhibitors with good properties [15]. PeptiDream utilizing Professor Suga’s mRNA display technology [16] are generating up to trillion-member...
libraries of larger macrocycles mimicking cyclosporin. These peptides contain a combination of natural, unnatural, and N-methyl amino acids and exhibit good physicochemical properties and membrane permeability [17]. Ra Pharmaceuticals also uses a mRNA display technology developed by Jack Shoatzac to generate very large libraries of macrocycles containing unnatural amino acids. They recently presented on their discovery of potent antagonists of mcl-1 and Ras with good cell permeability [18].

1.5 MODIFICATION OF PEPTIDES TO PROMOTE STABILITY AND CELL ENTRY

The recent focus on another class of macrocycles, containing multiple disulfides, has generated a lot of excitement in maintaining the stability and membrane permeability of the cyclotide kalata B1, or the knottins (the uncyclized version of cyclotides), in order to create potent peptide drugs. David Craik and colleagues at Cyclotide are systematically exchanging the various loops present on cyclotides with sequences that have important biological function [19]. Recently, the introduction of a myelin oligodendrocyte glycoprotein sequence into a cyclotide resulted in a potent peptide in preventing disease progression in a mouse model of MS [20]. Protagonist is taking advantage of the oral stability of the disulfide-rich peptides for local gut delivery of IL6R antagonists for the treatment of irritable bowel disease (IBD). Moreover, novel technologies developed for the rapid generation and screening of extremely large libraries of knottins and cyclotides will undoubtedly have a major impact on this class of peptide therapeutics. Of note is the Intein-based technology from Julio Camarero capable of introducing unnatural amino acids to facilitate screening [21]. Sutro and MitiBio also have very sophisticated and efficient biosynthetic methods to generate very large libraries.

Finally, Verdine and Wollensky and colleagues [22, 23] as well as the investigators at Aileron Therapeutics have developed a novel stapling technology that imparts stability and membrane permeability to alpha helical structure. Using this technology, Aileron Therapeutics were able to discover very potent dual MDM2/MDMx antagonists with low nanomolar activity in cells and excellent pharmacokinetic properties, resulting in excellent antitumor activity in a mouse xenograft model [24]. Even more interesting is the extended efficacy ATSP-7041 exhibits in cells. While the small molecule MDM2 antagonist showed activity over 24h, ATSP-7041 was still active beyond 48 hours in the same experiment. This is due to the fact that once the peptide enters the cell, the major elimination pathway is through enzymatic catabolism. Not only can stability be tuned for circulating half-life, it can also be tuned to withstand cellular catabolism to lengthen the desired efficacy. This could offer a significant advantage over (small) molecules that passively diffuse through the cell membrane. Additionally, using the same technology, a GHRH antagonist with much extended half-life was discovered and is currently in Phase I clinical trial [25].
1.6 TARGETING PEPTIDES TO SPECIFIC CELLS

One of the greatest challenges in drug discovery is the safety of therapeutics. Main reasons for diminished safety are selectivity against the target and tissue/cell specificity. If one could direct a therapeutic to only the site of pathology, then the therapeutic window of the agent increases and correspondingly decreases the side effects. Peptides, due to their specificity against receptors, are perfect candidates to be able to home into one type of cell/tissue versus another. There has been a tremendous amount of progress in identifying homing peptides (cell-penetrating as well as nonpenetrating) that can then be conjugated to a cargo to deliver it to a specific organ [26].

In vivo phage display by Pasqualini and colleagues marked the discovery of the first homing peptide that was able to selectively target the blood vessel of brain and kidney [27]. Since then a number of peptides have been identified that target many other tissues [28]. Arap and colleagues were then the first to perform phage display in humans and discovered a homing peptide to IL11Ra that expresses over 100-fold more on prostate cancer cells versus normal cells [29, 30]. Arrowhead Research is currently in Phase I proof of targeting with a peptide drug conjugate utilizing this homing peptide. Recently, Wen et al., at the Dana Farber, published their first Phase I study result on GRN1005, a peptide drug conjugate that targets the low-density lipoprotein-related protein-1, which mediates blood brain barrier transcytosis. GRN1005 successfully crosses the BBB and delivers its cargo [31].

1.7 FORMULATIONS TO IMPROVE PROPERTIES

While the above advances have and will have significant impact, the ability to administer peptides by the oral route will truly allow them to compete with small molecules and biologics as first line therapies. The majority of advances in this area have been the result of very interesting formulation strategies. A number of companies, including ArisGen, Axcess, Chiasma, Emisphere Tech., Enteris Pharmaceuticals, Lipocine, and Merlion Pharmaceuticals, have had successes in enhancing the oral bioavailability of some peptide therapeutics. They employ a combination of stabilizers, absorption enhancers, and carriers to achieve this. The main mode of absorption is through the paracellular space. However, the bioavailability of the peptides formulated remains relatively low.

While significant, cyclosporin remains the only marketed peptide drug that is administered orally and absorbed into the systemic environment. Learning from nature and systematic studies on macrocyclic peptides will have a tremendous impact in discovering peptide drugs with inherent oral bioavailability that could then be enhanced through formulation to achieve bioavailabilities, which would compete with small molecules. As mentioned earlier, PeptiDream and Ra Pharmaceuticals are generating large libraries of macrocyclic peptides mimicking the core structure of cyclosporin. Ensemble therapeutics are generating small macroyclic structures with molecular weights between 500 and 1500 and have already identified an orally
bioavailable IL17 R antagonist. Professors Horst Kessler and Locky are doing the first systematic studies on small cyclic peptides to understand the effect of hydrogen bonding and structure on bioavailability [32, 33]. Their work will undoubtedly form the basis of rational designs of orally active peptide drugs.

In conclusion, the great technological advances over the last two decades are well poised to have a major impact on revolutionizing the field of peptide therapeutics. For the first time, tools are available to create stable, cell permeable, long lasting, and orally bioavailable peptides, allowing them to compete with small molecule drugs and biologics, and thus become first line therapies for many diseases with unmet medical needs.

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