Peptide therapeutics: current status and future directions☆

Keld Fosgerau and Torsten Hoffmann

Zealand Pharma A/S, Smedeland 36, Glostrup, 2600 Copenhagen, Denmark

Peptides are recognized for being highly selective and efficacious and, at the same time, relatively safe and well tolerated. Consequently, there is an increased interest in peptides in pharmaceutical research and development (R&D), and approximately 140 peptide therapeutics are currently being evaluated in clinical trials. Given that the low-hanging fruits in the form of obvious peptide targets have already been picked, it has now become necessary to explore new routes beyond traditional peptide design. Examples of such approaches are multifunctional and cell penetrating peptides, as well as peptide drug conjugates. Here, we discuss the current status, strengths, and weaknesses of peptides as medicines and the emerging new opportunities in peptide drug design and development.

Introduction

More than 7000 naturally occurring peptides have been identified, and these often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives [1–4]. In general, peptides are selective and efficacious signaling molecules that bind to specific cell surface receptors, such as G protein-coupled receptors (GPCRs) or ion channels, where they trigger intracellular effects. Given their attractive pharmacological profile and intrinsic properties, peptides represent an excellent starting point for the design of novel therapeutics and their specificity has been seen to translate into excellent safety, tolerability, and efficacy profiles in humans. This aspect might also be the primary differentiating factor of peptides compared with traditional small molecules. Furthermore, peptide therapeutics are typically associated with lower production complexity compared with protein-based biopharmaceuticals and, therefore, the production costs are also lower, generally approaching those of small molecules. Thus, in several ways, peptides are in the sweet spot between small molecules and biopharmaceuticals.

Naturally occurring peptides are often not directly suitable for use as convenient therapeutics because they have intrinsic weaknesses, including poor chemical and physical stability, and a short circulating plasma half-life. These aspects must be addressed for their use as medicines. Some of these weaknesses have been successfully resolved through what we term the 'traditional design' of therapeutic peptides as described below (see Fig. 1 for a full SWOT analysis of peptides as therapeutics). Besides traditional peptide design, a range of peptide technologies has been emerging that represent the opportunities and future directions within the peptide field. These include multifunctional and cell penetrating peptides, as well as peptide drug conjugates and technologies focusing on alternative routes of administration. Here, we present a chain of thoughts leading to the conclusion that peptides offer enormous growth potential as future therapeutics.

Peptide drug market

During the past decade, peptides have gained a wide range of applications in medicine and biotechnology, and therapeutic peptide research is also currently experiencing a renaissance for commercial reasons. For example, the peptide-based medicine Lupron™ from Abbott Laboratories for the treatment of prostate cancer and more, achieved global sales of more than US$2.3 billion in 2011 [5]. In addition, Lantus™ from Sanofi (which is really at the border between a peptide drug and a small biopharmaceutical) reached sales of US$7.9 billion in 2013. Currently, there are more than 60 US Food and Drug Administration (FDA)-approved peptide medicines on the market and this is expected to grow significantly, with approximately 140 peptide drugs currently in clinical trials and more than 500 therapeutic peptides in preclinical

Corresponding author: Hoffmann, T. (tho@zealandpharma.com)

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/

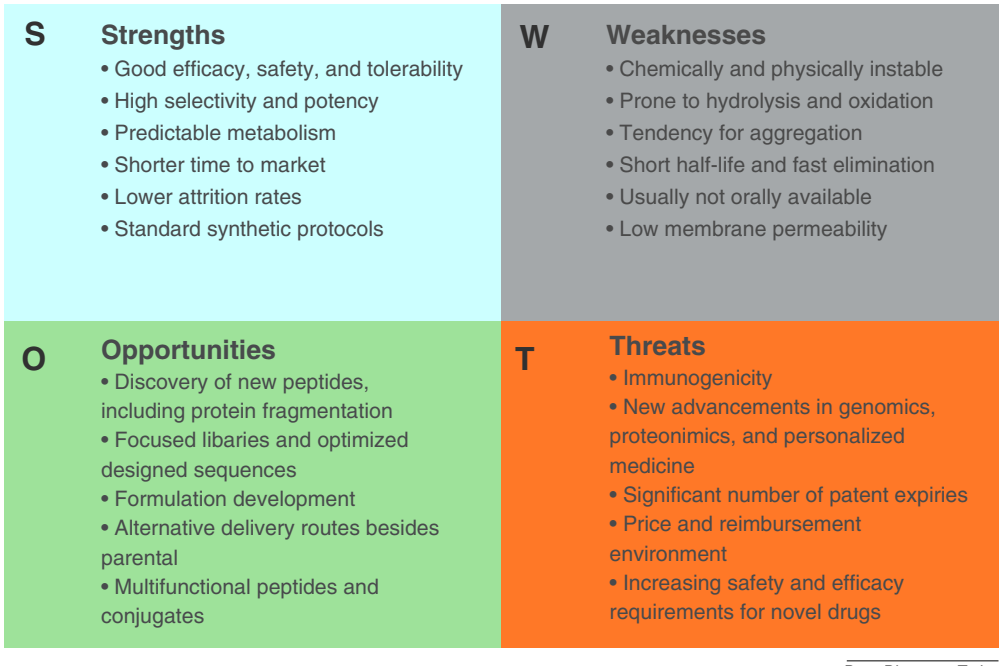

FIGURE 1

Analysis of the strengths, weaknesses, opportunities, and threats (SWOT) of naturally occurring peptides in their use as therapeutics as seen from our point of view.

development. An overview was presented in a recent review by Kaspar and Reichert [5].

In terms of value, the global peptide drug market has been predicted to increase from US$14.1 billion in 2011 to an estimated US$25.4 billion in 2018, with an underlying increase in novel innovative peptide drugs from US$8.6 billion in 2011 (60%) to US$17.0 billion (66%) in 2018 [6]. The most recent example of a novel peptide drug class is the group of glucagon-like peptide-1 (GLP-1) agonists for the treatment of type 2 diabetes mellitus (T2DM), which reached total sales of over US$2.6 billion in 2013, with Victoza™, the most prominent member of the class, reaching blockbuster status. A status of the top-selling peptide therapeutics was given in a recent report from Transparency Market Research [6].

The main disease areas currently driving the therapeutic use of peptide drugs are metabolic diseases and oncology. The former can be characterized by the epidemic growth in both obesity and T2DM, the latter by a rising mortality and need for chemotherapy replacement, as well as cancer supportive care. The use of peptide therapeutics in the treatment of diabetes and obesity is probably why North America currently represents the largest share of the peptide drug market, with the Asian market expected to have the largest growth. The movement of the pharmaceutical industry into rare diseases and orphan drugs has also been extended to peptides, and marketed examples in this area include teduglutide, a GLP-2 receptor 2 agonist for short bowel syndrome, and pasireotide, a somatostatin receptor agonist for the treatment of Cushing's syndrome. Moreover, there seems to be a current trend towards the disease areas of infectious diseases and inflammation, where several peptides are undergoing clinical testing.

Currently, most peptide drugs are administered by the parental route and approximately 75% are given as injectables. However, alternative administration forms are gaining increasing traction, including oral, intranasal, and transdermal delivery routes, according to the respective technology developments [6]. One example of an alternative administration route evaluated for application to peptides is the transbuccal delivery via the combination of gold nanoparticles (Midatech) and the PharmFilm™ (Monosol Rx) technology. Accordingly, Midasol Therapeutics is currently carrying out clinical development of a transbuccal delivery system that utilizes insulin-passivated gold glyconanoparticles [7]. Another example is the TopAct™ technology platform from ActoGeniX, which might enable oral delivery of peptides directly expressed in the gastrointestinal tract [8].

The use of alternative administration forms could also enable greater usage of peptide therapeutics in other disease areas, such as inflammation, where topical administration of peptides could be the basis for highly efficacious novel treatments. Although not within the scope of this article, peptides are often excellent biomarkers and, therefore, can also be used for diagnostic purposes [9]. Finally, peptides have also found an application as vaccines.

Traditional peptide technologies

Peptides have evolved as highly potent signal transduction molecules, exerting powerful physiological effects. As illustrated in our SWOT analysis (Fig. 1), they are generally characterized by a relatively short circulating plasma half-life, as well as suboptimal physical and chemical properties for their use as medicines. Consequently, traditional rational design of peptide therapeutics has focused on techniques to mitigate these weaknesses, as outlined in Fig. 2.

Rational design of peptide therapeutics

Rational design can start with a known crystal structure of the peptide giving the secondary and tertiary structure. Then, via

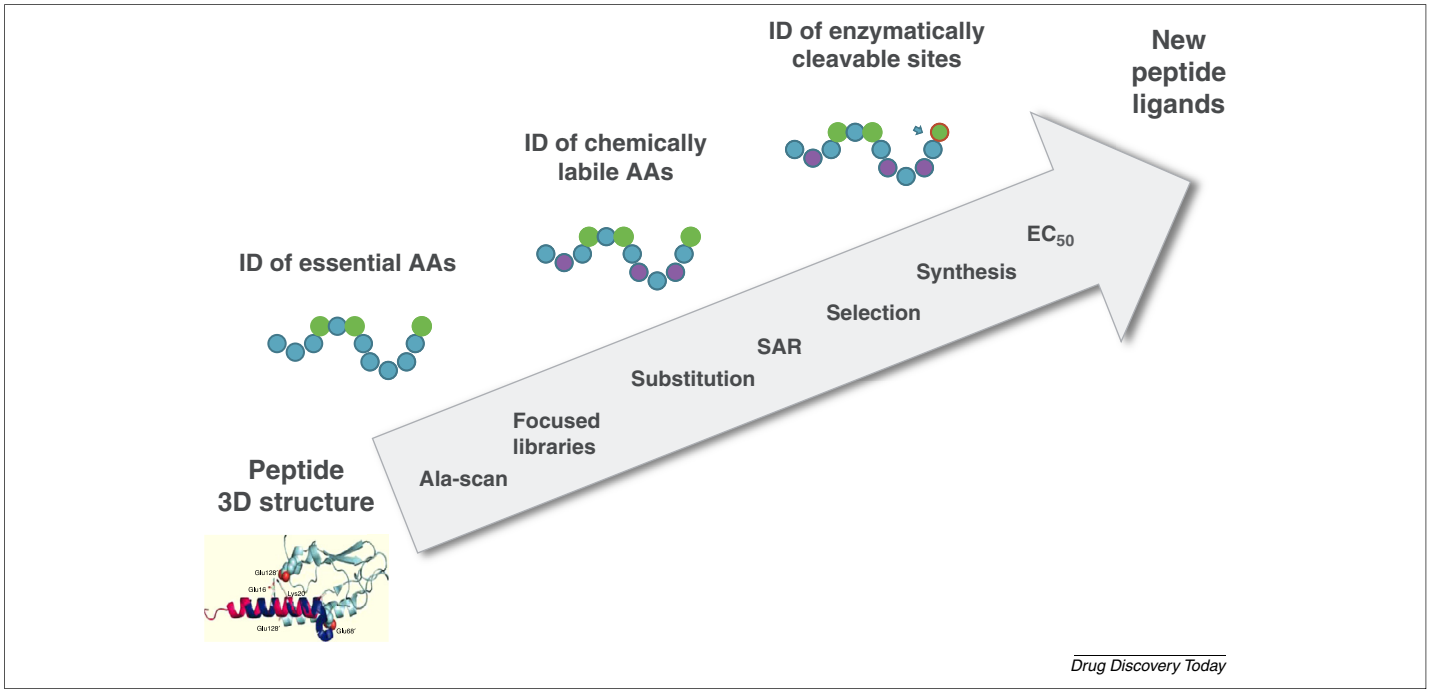

FIGURE 2

The traditional structure-based design strategies that are used in peptide drug discovery. This includes substitution of amino acids (AA), and the building of structure–activity relations (SAR) via elements such as an alanine (Ala) scan and estimation of EC_{50} (half maximal effective concentration).

input from various analyses, such as alanine substitutions (Ala-scan), and small focused libraries, the structure–activity relation (SAR) is built in sequential steps that lead to the identification of essential amino acids and also sites for possible substitution. In this process, and especially when liquid drug formulations are the desired final product, it is an important step to identify amino acids that are chemically labile and prone to events such as isomerization, glycosylation, or oxidation, which should be avoided [10].

Another important aspect in rational peptide drug design is to improve the physicochemical properties of natural peptides, which often have a tendency to aggregate and are sometimes poorly water soluble [11]. Chemical design strategies to avoid aggregation include the corruption of hydrophobic patches, which can be achieved by means of substitutions or *N*-methylation of particular amino acids. If solubility issues exist for a certain peptide drug candidate, the common focus area is on charge distribution and the isoelectric point (p*I*) of the peptide, in relation to the pH of the desired formulation of the final product.

The physicochemical properties of peptides can also be improved by the introduction of stabilizing α-helixes, salt bridge formations, or other chemical modifications, such as lactam bridges. Modifications introduced via rational design to improve the physicochemical properties of a given peptide must match the overall desired pharmacological and pharmacokinetic properties of the peptide therapeutic. The use of predictive IT software tools for the facilitation of such rational peptide design is expected to increase in the future as these tools become increasingly user friendly.

The challenges that the intrinsic physicochemical properties of peptides represent for their use as medicines have, in some cases, led to suboptimal solutions for patients and physicians. An example is the therapeutic use of glucagon for the treatment of severe hypoglycemia in diabetes. Currently, the only marketed glucagon rescue kit for the treatment of unconscious, hypoglycemic patients, contains a lyophilized peptide powder in a sterile vial that needs to be carefully reconstituted by gentle agitation 30 times before it can be administered subcutaneously to the unconscious patient. This is a cumbersome process in what is already a stressful situation (http://www.humalog.com/Pages/glucagon-severe-low-blood-sugar.aspx).

There is a general notion that second-generation peptide medicines optimized for therapeutic use via rational design will lead to more user-friendly products. An example of such an approach is the invention and design of a glucagon analog that is stable in a liquid dosage form and suitable for application as a ready-to-use rescue pen [12]. Furthermore, such products can be used as part of a closed-loop dual chamber artificial pancreatic device that can deliver both insulin and glucagon, where the therapeutics would be centrally released by an automatic pump and coupled to a continuous blood glucose sensor. With the rapid development and further miniaturization of devices, pumps, and IT controlled sensor feedback systems, it is envisaged that pulsatile delivery systems could be developed that would enable smart delivery of peptides. These medicines might apply and mimic the rapid on- and off-set of natural peptides, as in the glucagon example above.

Approaches for plasma half-life extension

In general, natural peptides have a relatively short circulating plasma half-life and, thus, several techniques for half-life extension have been developed. A first-line approach is to limit the enzymatic degradation of the peptide through identification of possible molecular cleavage sites followed by substitution of

the relevant amino acids (Fig. 2). Protection against enzymatic cleavage can also be obtained through enhancement of the secondary structure of the peptides (i.e. folding). This approach includes insertion of a structure inducing probe (SIP)-tail, lactam bridges, and stapling or cliping of peptide sequences, or by cyclization [13–16]. Biotechnology companies working in this research field include Pepscan and Aileron Therapeutics.

Several strategies use binding to the circulating protein albumin as a vehicle to obtain half-life extensions that lead to peptide therapeutics that have to be administered less frequently, in some cases up to once weekly. These strategies include peptide acylation (as seen in the GLP-1 agonist Victoza [17]), insertion of albumin-binding peptide elements in the peptide backbone, or conjugation to albumin-binding antibody fragments (AlbudAb™ technology, [18]). Polyethylene glycol (PEG)-ylation has been used to limit globular filtration and thereby increasing plasma half-life by limiting the elimination of peptides [19]. However, because of increased safety and tolerability concerns relating to the use of PEG as a component of an injectable therapeutic, PEGylation has become a less preferred choice (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/11/WC500135123.pdf). Finally, much can be achieved in terms of extending the plasma half-life of a peptide (and also its chemical stability) by means of formulations. Recently, an ingenious formulation approach was developed by Intarcia [20] using an implantable device that delivers peptide therapeutics from a dry reservoir for up to 1 year through an osmotic pump mechanism. This innovative approach opens an entirely new avenue for the delivery of peptides as medicines, addressing patient convenience and compliance through the successful design of a most-elegant delivery solution. Before the Intarcia solution, another earlier example of such an approach is the leuprolide acetate implant (Viadur™) for the palliative treatment of advanced prostate cancer.

Emerging peptide areas and technologies

There is a large pool of natural peptides, some of which represent excellent starting points for therapeutics. In the metabolic area, for example the gut, the microbiome has received much interest because it is rich in diverse bacteria that could give rise to the identification of new peptides from protein fragments, degradation products, or signaling molecules [21]. We are convinced that ongoing microbiome research will help to enrich significantly the opportunities for peptide therapeutics in metabolic diseases.

However, for innovative peptide drug development, chemists must look beyond traditional peptide technologies. Among the emerging technologies in the field are multifunctional peptides representing more than one pharmacological activity, such as dual or even triple agonism. This approach makes sense based on information from genomics. Thus, it is evident that knockout animals, where only a single gene is deleted, often present with no distinct phenotype. Also, despite broad industrial efforts in the GPCR field that have led to the successful identification of several selective agonists and antagonists in clinical development, only a few ligands resulted in approved medicines. These lessons point to the redundancy in biological systems and favor multitarget approaches for the development of medicines. Another aspect of applying a polypharmacology approach is the possibility of more individualized and personalized treatment of differentiated patient groups.

Current multifunctional peptides in development include antimicrobial peptide drug candidates that have additional biological functions, such as immune stimulation and wound healing. Also, the trend towards multifunctional peptides can be seen in the GLP-1 agonist field, which represents an established drug class with several products, including Byetta™ (exenatide), Bydureon™ (exenatide), Victoza™ (liraglutide), Lyxumia™ (lixisenatide), and most recently Tanzeum™ (albiglutide), being

TABLE 1

GLP-1 agonists currently in clinical or preclinical testing[a]

Company	Peptide name	Development stage	Target	Dosing regimen
Lilly	Cpd86	Preclinical	GLP-1/GIP	SC, once daily
Zealand Pharma	ZPGG-72	Preclinical	GLP-1/GLP-2	SC, once daily
	ZP3022	Preclinical	GLP-1/CCKB	SC, once daily
Prolor (Opko Biologics)	MOD-6030	Preclinical	GLP-1/GCG	SC, once weekly
Zealand Pharma	ZP2929	Phase I	GLP-1/GCG	SC, once daily
Hamni Pharmaceuticals	HM12525A	Phase I	GLP-1/GCG	SC, once weekly
Diartis Pharmaceuticals	VSR859	Phase I	GLP-1	SC, once monthly
Novo Nordisk	NN9926	Phase I	GLP-1	Oral, long acting
TransTech Parma	TTP273/TTP054	Phase II	GLP-1	Oral
Zydus-Cadila	ZYOG1	Phase I	GLP-1	Oral
Roche	MAR709	Phase II	GLP-1/GIP	SC, once daily
Eli Lilly	TT401	Phase II	GLP-1/GCG	SC, once weekly
Hamni Pharmaceuticals	HM11260C	Phase II	GLP-1	SC, once weekly
PhaseBio Pharmaceuticals	PB1023	Phase II	GLP-1	SC, once weekly
Eli Lilly	Dulaglutide	Phase III	GLP-1	SC, once weekly
Novo Nordisk	Semaglutide	Phase III	GLP-1	SC, once weekly
Intarcia	ITCA	Phase III	GLP-1	SC, once yearly

[a] *Abbreviation*: SC subcutaneous.

introduced to the market with great commercial success. Looking across clinical and preclinical pipelines, it is evident that several companies have focused on the development of GLP-1 dual and even triple agonists for a more diversified and personalized treatment of T2DM and/or obesity (Table 1). It is also evident from Table 1 that, in addition to multifunctional peptides, there is a focus on improved patient convenience and compliance and, therefore, strategies towards less-frequent dosing, or even oral administration of GLP-based drugs, being pursued in clinical development.

Examples of other peptide compounds or modules that have been combined with GLP-1 agonism include glucagon (GCG) [22,23], glucose-dependent insulinotropic peptide (GIP) [24], cholecystokinin B (CCKB) [25], and glucagon-like peptide 2 (GLP-2) [26]. The most clinically advanced multifunctional peptides are GLP-1–GIP and GLP-1–GCG dual agonists, which are currently being investigated in clinical proof-of-concept studies in patients who are overweight and have diabetes. The GLP-1–GCG dual agonists (some of which are modulated over the natural dual-acting gut peptide oxyntomodulin) are expected to provide a greater weight loss in overweight patients with T2DM compared with a pure GLP-1 agonist, via a GCG-derived increase in energy expenditure [27]. Another example is the GLP-1–CCKB dual agonists [25] where the addition of CCKB (gastrin) agonism to the GLP-1 action is expected to enhance the pancreatic β cell function, which in turn might aid in minimizing or preventing disease progression in T2DM. These examples illustrate how the addition of a second activity to the established effects of GLP-1 could lead to more individualized medical solutions with increased efficacy (Fig. 3).

Chemical strategies for the design of multifunctional peptides can include a hybrid of two peptides being bound together like modules either directly or via a linker, or chimeras where the second pharmacological activity is 'designed in' to an existing peptide backbone (Fig. 3). Most recently, GLP-1–GIP–GCG triple

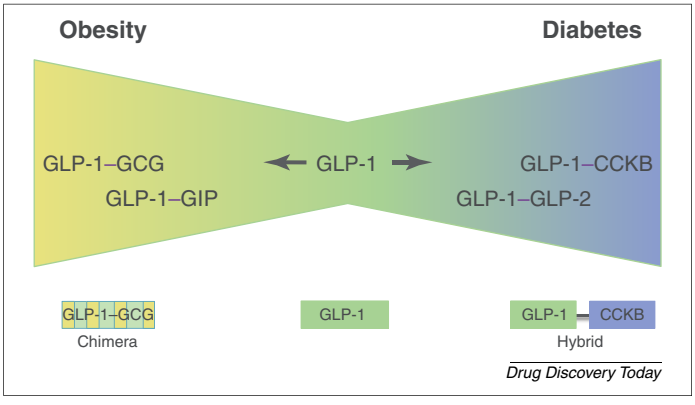

FIGURE 3

Multifunctional peptides. The figure illustrates how the diversification of GLP-1-based peptides into multifunctional peptides could occur for fine-tuned pharmacology towards obesity or disease prevention in type 2 diabetes mellitus. These multifunctional peptides could include a hybrid of two peptides being bound together like modules either directly or via a linker, or chimeras where the second pharmacological activity is 'designed in' to an existing peptide backbone. *Abbreviations*: CCKB, cholecystokinin B; GCG, glucagon; GIP, glucose-dependent insulinotropic peptide; GLP-1/2, glucagon-like peptide 1/2.

incretin agonists have been described in the patent literature as potent antidiabetic and antiobesity agents, having shown strong, beneficial effects in rodents [28,29], and even peptides acting on four different receptors (quadruples) are, in our experience, chemically feasible, but have not yet emerged in the public domain.

The development of multifunctional peptides could present a challenge in the sense that the prediction of the *in vivo* outcome for the drug candidates is more complex with dual target pharmacology versus single target pharmacology. One challenging aspect of the translation from *in vitro* to *in vivo* effects is the potential biased signaling that might arise from novel ligands aimed at two or more receptors [30]. In addition, the translation of results from animal models to human situations might be associated with greater risk for multifunctional peptides compared with single receptor peptides, because the uncertainty from two or more targets is multiplied. In the antibody field, similar challenges have been observed in the development of bispecific antibodies for the treatment of cancer. For these reasons, it is relevant to expect that multifunctional peptides might arise mainly from established paradigms, as observed in the GLP-1 field, more than from completely novel peptide combinations.

Most peptide therapeutics are injectables and there are only a few oral peptide drugs in existence, cyclosporine (Neoral™) and desmopressin (Minirin™) being prominent examples. However, it is expected that the market will see an increase in orally bioavailable peptides in development because they offer greater convenience for patients. The challenges relating to development of oral peptides include acidic and enzymatic degradation of the molecules in both the gastrointestinal tract, and when crossing the intestinal mucosa via either active transport or passive diffusion. Therefore, chemical strategies in the design of peptides for oral administration include the previously mentioned stabilizing of the secondary structures, such as stapled peptides, building hydrophobic faces, cyclization, *N*-methylation, and establishment of intramolecular hydrogen bonds. Various biotechnology companies are active in this field of peptide drug research, including Ra Pharma, Peptidream, and Cyclogenix (cyclic peptides), and Bicycle Therapeutics (phage display using chemical ligation libraries).

Formulary aspects and permeability enhancers are also important. The question applicable to orally bioavailable peptide drugs is whether the molecular changes introduced through the above-mentioned derivatizations hamper the *in vivo* efficacy of the therapeutics. Another point of relevant consideration in relation to the development of orally available peptide therapeutics is to what extent an injectable medicine is really inconvenient for patients. New and more patient-friendly injection technologies are constantly being developed, including smaller needles and even more convenient devices, which should also be taken into account in the context of the severity of the disease being treated. It is our belief that injectable peptide medicines will remain the preferred solution over orally administered peptides, especially for patient populations who are already using injectables. An example would be patients with diabetes treated with insulin.

Native peptides in general do not cross cell membranes, which has previously limited their therapeutic use to extracellular targets. However, over recent years, technologies have been invented for the insertion of membrane permeability elements, including so-called 'cell penetrating peptides', such as penetratin or the

transcription *trans*-activating (TAT) sequences (http://cedarburghauserpharma.com/wp-content/uploads/peptide-drug-conjugates-specchem1.pdf), making it possible to reach intracellular targets with peptides to some degree. One challenge of cell penetrating peptides, which is likely to be also shared by orally bioavailable peptides, is a probable loss of efficacy because usually only a fraction of the peptide drug reaches the target. Another important aspect to consider when developing cell penetrating peptides is whether their molecular properties resemble those of small molecules more, including lower specificity and, therefore, greater safety risk because of an increased volume of distribution. In other words, making peptides cell penetrant or orally bioavailable might tip the SWOT analysis (Fig. 1) towards that of a small molecule.

Finally, we point to conjugation of peptides with, for example, small molecules, oligoribonucleotides, or antibodies as new possible avenues to pursue the development of novel peptide therapeutics with improved efficacy and safety properties. In oncology, for example, this approach has gained must interest, resulting in more than 20 peptide conjugates being evaluated in clinical trials (http://cedarburghauserpharma.com/wp-content/uploads/peptide-drug-conjugates-specchem1.pdf). Although peptides in clinical testing in the oncology area in general have experienced a relatively high level of attrition, this conjugation principle has been exemplified by the combination of a neurotensin 1 (NT1) receptor peptide agonist conjugated to a radioactive ligand for the treatment of pancreatic cancer. Via this conjugate, the peptide component ensures targeted delivery of the radioligand to the NT1 receptor expressed at the relevant site, enabling a high local concentration of the chemotherapeutic agent. As a consequence, other parts of the body are also less exposed, which in turn is expected to result in an improved safety margin, and potential higher and more efficacious dosing of the anticancer agent [31]. In antibody–peptide conjugates, the antibody part might take the role as the targeting entity, whereas the peptide is the effector part. Pursuing the conjugation route for multifunctional peptides might not only result in an attractive therapeutic potential, but also present challenges in the form of a relatively complex drug development path, including increased regulatory requirements and more complex production of the pharmacologically active ingredient. Biotechnology companies working with peptide conjugates include Angiochem and 3B Pharmaceuticals, among others.

Concluding remarks

Peptides have gained increased interest as therapeutics during recent years. More than 60 peptide drugs have reached the market for the benefit of patients and several hundreds of novel therapeutic peptides are in preclinical and clinical development. The key contributor to this success is the potent and specific, yet safe, mode of action of peptides. We believe that the future development of peptide drugs will continue to build upon the strengths of naturally occurring peptides, with the application of traditional rational design to improve their weaknesses, such as their chemical and physical properties. We also expect that emerging peptide technologies, including multifunctional peptides, cell penetrating peptides and peptide drug conjugates, will help broaden the applicability of peptides as therapeutics. These new peptide technologies include alternative administration routes beyond the predominantly parental injection route and conjugates of peptides to antibodies or to small molecules. We foresee that medicinal peptide chemistry could be applied increasingly in a modular fashion, comparable to microelectronics or LEGO^{TM} bricks. The intelligent assembly of known modules with unique properties and functions could lead to the construction of multifunctional molecular medicines with improved efficacy, pharmacokinetic properties, and targeted delivery. The analogy to LEGO^{TM} bricks is a good one because the possible combinations of amino acids into peptides are almost unlimited, for example, a combination of any of the 20 naturally occurring amino acids built into a 20-mer would alone give $20^{20} = 1.05 \times e^{26}$ possibilities. Taking all of the above into account, we are convinced that peptides offer enormous growth potential as future therapeutics for the treatment of unmet medical needs.

Acknowledgements

The essential contributions of our Zealand Pharma chemistry colleagues Ditte Riber, Pernille T. Shelton, Oliver Demmer, Jakob L. Tolborg, Marie Skovgaard, and Bjarne Due Larsen were highly appreciated. We acknowledge the Zealand Pharma Executive Committee, especially Hanne L. Hillman and David Solomon, for dedicated support and guidance, and Maria Juul Kolding for technical support.

References

1 Padhi, A. *et al.* (2014) Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects. *Tuberculosis* 94, 363–373
2 Buchwald, H. *et al.* (2014) Effects on GLP-1, PYY, and leptin by direct stimulation of terminal ileum and cecum in humans: implications for ileal transposition. *Surg. Obes. Relat. Dis.* http://dx.doi.org/10.1016/j.soard.2014.01.032
3 Giordano, C. *et al.* (2014) Neuroactive peptides as putative mediators of antiepileptic ketogenic diets. *Front. Neurol.* 5, 63
4 Robinson, S.D. *et al.* (2014) Diversity of conotoxin gene superfamilies in the venomous snail, *Conus victoriae*. *PLOS ONE* 9, e87648
5 Kaspar, A.A. and Reichert, J.M. (2013) Future directions for peptide therapeutics development. *Drug Discov. Today* 18, 807–817
6 Transparency Market Research (2012) *Peptide Therapeutics Market: Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2012–2018*. Transparency Market Research
7 Sheridan, C. (2012) Proof of concept for next-generation nanoparticle drugs in humans. *Nat. Biotechnol.* 30, 471–473
8 Steidler, L. *et al.* (2009) Actobiotics as a novel method for cytokine delivery. *Ann. N. Y. Acad. Sci.* 1182, 135–145
9 Schulte, I. *et al.* (2005) Peptides in body fluids and tissues as markers of disease. *Expert Rev. Mol. Diagn.* 5, 145–157
10 Manning, M.C. *et al.* (2010) Stability of protein pharmaceuticals: an update. *Pharm. Res.* 27, 544–575
11 Hamley, I.W. (2007) Peptide fibrillization. *Angew. Chem. Int. Ed. Engl.* 46, 8128–8147
12 Riber, D. *et al.* (2013) A novel glucagon analogue, ZP-GA-1, displays increased chemical and physical stability in liquid formulation. *Diabetes* 62, A99–A172
13 Larsen, B. *et al.* Zealand Pharma A/S. Pharmacologically active peptide conjugates having a reduced tendency towards enzymatic hydrolysis. US 6,528,486 B1, WO 99/46283
14 Timmerman, P. *et al.* (2009) Functional reconstruction of structurally complex epitopes using CLIPSTM technology. *Open Vaccine J.* 2, 56–67
15 Houston, M.E., Jr *et al.* (1996) Lactam bridge stabilization of alpha-helices: the role of hydrophobicity in controlling dimeric versus monomeric alpha-helices. *Biochemistry* 35, 10041–10050

16 Sim, S. *et al.* (2012) Directional assembly of alpha-helical peptides induced by cyclization. *J. Am. Chem. Soc.* 134, 20270–20272
17 Knudsen, L.B. (2010) Liraglutide: the therapeutic promise from animal models. *Int. J. Clin. Pract. Suppl.* 4–11
18 Bao, W. *et al.* (2013) Novel fusion of GLP-1 with a domain antibody to serum albumin prolongs protection against myocardial ischemia/reperfusion injury in the rat. *Cardiovasc. Diabetol.* 12, 148
19 Delgado, C. *et al.* (1992) The uses and properties of PEG-linked proteins. *Crit. Rev. Ther. Drug Carr. Syst.* 9, 249–304
20 Henry, R.R. *et al.* (2014) Continuous subcutaneous delivery of exenatide via ITCA 650 leads to sustained glycemic control and weight loss for 48 weeks in metformin-treated subjects with type 2 diabetes. *J. Diabetes Complicat.* 28, 393–398
21 Hattori, M. and Taylor, T.D. (2009) The human intestinal microbiome: a new frontier of human biology. *DNA Res.* 16, 1–12
22 Pocai, A. *et al.* (2009) Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. *Diabetes* 58, 2258–2266
23 Fosgerau, K. *et al.* (2011) The new glucagon-GLP-1 dual agonist ZP2929 in combination with long-acting insulin improves glycemic control without causing weight loss in db/db mice. *Diabetes* 60, A408–A442
24 Finan, B. *et al.* (2013) Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci. Transl. Med.* 5, 209ra151
25 Fosgerau, K. *et al.* (2013) The novel GLP-1-gastrin dual agonist, ZP3022, increases beta-cell mass and prevents diabetes in db/db mice. *Diabetes Obes. Metab.* 15, 62–71
26 Just, R. *et al.* (2014) The novel GLP-1-GLP-2 dual agonist ZP-GG-72 increases intestinal growth and improves insulin sensitivity in DIO mice. *Diabetes* 63, A1–A102
27 Pocai, A. (2012) Unraveling oxyntomodulin, GLP1's enigmatic brother. *J. Endocrinol.* 215, 335–346
28 Gault, V.A. *et al.* (2013) A novel glucagon-like peptide-1 (GLP-1)/glucagon hybrid peptide with triple-acting agonist activity at glucose-dependent insulinotropic polypeptide, GLP-1, and glucagon receptors and therapeutic potential in high fat-fed mice. *J. Biol. Chem.* 288, 35581–35591
29 DiMarchi, R.D. *et al.* Hoffmann–La Roche Inc. Analogs of glucagon exhibiting GIP receptor activity. PCT/US2013/046229, WO/2013/192130
30 Zhou, L. and Bohn, L.M. (2014) Functional selectivity of GPCR signaling in animals. *Curr. Opin. Cell Biol.* 27, 102–108
31 Okarvi, S.M. (2008) Peptide-based radiopharmaceuticals and cytotoxic conjugates: potential tools against cancer. *Cancer Treat. Rev.* 34, 13–26