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Peptides as a platform for targeted therapeutics for cancer: peptide–drug conjugates (PDCs)

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Peptides can offer the versatility needed for a successful oncology drug discovery approach. Peptide–drug conjugates (PDCs) are an emerging targeted therapeutic that present increased tumour penetration and selectivity. Despite these advantages, there are still limitations for the use of peptides as therapeutics exemplified through their slow progression to get into the clinic and limited oral bioavailability. New approaches to address these problems have been studied and successfully implemented to enhance the stability of peptides and their constructs. There is great promise for the future of PDCs with two molecules already on the market and many variations currently undergoing clinical trials, such as bicycle-toxin conjugates and peptide–dendrimer conjugates. This review summarises the entire process needed for the design and successful development of an oncology PDC including chemical and nanomaterial strategies to enhance peptide stability within circulation, the function of each component of a PDC construct, and current examples in clinical trials.

Key learning points

1. A variety of methods to address peptide chemical and enzymatic stability can be implemented.
2. The design of a PDC requires understanding of the mechanism of action intended and hence environmental stimuli (pH, GSH and enzymes).
3. The function and rationale behind the design of each component of a PDC.
4. The stability of the PDC construct can be improved through the use of nanomaterials.
5. Bicycle-toxin conjugates (BTCs) and peptide-dendrimer conjugates are emerging constructs that fall under the umbrella term PDCs.

Introduction

Traditionally, research within drug discovery falls into two groups: small molecules (<500 Da) and biologics (>5000 Da).[1] Peptides are placed within the molecular weight range that is typically under-represented in the pharmaceutical company pipelines. A peptide is defined by the FDA as a polymer composed of less than 40 amino acids (500–5000 Da).[2] Over recent years, the research community is acknowledging the many advantages that peptides bring over small molecules and biologics. These include simpler design, ability to interact with underexplored targets, cheaper synthesis, decreased immunogenicity and enhanced tissue penetration.[1] To date in the U.S., Europe and Japan markets, there are more than 100 peptide drugs used to treat a range of diseases.[2] Financially, the peptide market is lucrative as it is estimated to be worth £11–16 billion annually by 2019.[2] However, there is still a significant challenge for the pharmaceutical industry to get peptides to market with many adopting greener peptide synthesis techniques at increased costs than traditional approaches.

Peptides can offer a multifunctional approach – in addition to being biologically active, they are excellent at transporting cargos to the desired targets. Their use within targeted therapeutics is an exciting area of research with great promise in the future with particular focus in, but not limited to, oncology. Witnessing the current success and investment into many antibody–drug conjugates (ADCs), the equivalent peptide–drug conjugates (PDCs) show promise for the future of the use of peptides within this setting. This review will highlight the excellence and limitations of peptides, their use in PDCs for advancing targeted cancer therapeutics and will consider how the specific tumour microenvironment can aid the design of a PDC. In addition, the review provides an examination of how

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the stability of PDCs in circulation can be enhanced through both chemical modifications and material science – a topic rarely discussed but extremely valuable to the successful development of new peptide drugs.

ADME & PK considerations for peptides

When considering therapeutic agents, it is crucial to analyse pharmacokinetic (PK) properties such as absorption, distribution, metabolism and excretion (ADME) as well as pharmacodynamic (PD) properties. Traditionally, PK properties of peptides tend to differ substantially from those of small drug molecules.[3] A significant limitation of peptides is their limited or non-existent oral bioavailability leading to administration through intravenous (IV) injection. One of the most convenient methods of administration is oral as it allows the patient to take the medication independently, unlike IV that often requires a clinical setting.

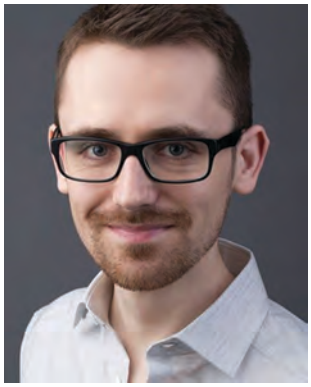
A small molecule is defined as 'drug-like' if it satisfies the criteria of Lipinski's Rule of 5 (Ro5). These rules focus on several fundamental factors including the molecular weight (<500 Da), ≤ 5 H-bond donors, ≤ 10 H-bond acceptors and $\log P$ (<5): molecules that satisfy the Ro5 are likely to be orally bioavailable.[4] Peptides do not fit into the Ro5 criteria due to their relatively large size (500–5000 Da) in comparison to small molecules (typically <500 Da). Despite peptides not satisfying the Ro5 criteria, the rule does not indicate that a peptide cannot become a drug as proven through many peptides on the market and in clinical trials.[4]

Bethany M. Cooper

Bethany M. Cooper received her MChem degree from the University of Leeds in 2018, having completed her 3rd year of study at Lubrizol Ltd, Hazelwood. On return to the University of Leeds her final year was spent under the supervision of Professor Steve Marsden. In 2019, she started her PhD studies at the University of Cambridge under the supervision of Professor David Spring and industrial supervisor Dr Maria Ölwegård-Halvarsson, where her research has focused on peptide stapling methodologies for use within therapeutics.

Jessica Iegre

Jessica Iegre was born in Italy and obtained her MSci in Medicinal Chemistry and Pharmaceutical Technology at the University of Pisa, Italy in 2013. The same year, she joined the AstraZeneca IMED Graduate programme in Göteborg, Sweden where she spent 2 years working across three different departments: medicinal chemistry, DMPK and computational chemistry. In 2015 she joined the Spring group at the University of Cambridge, and she obtained her PhD in chemical biology in 2019. Jessica is currently a Postdoctoral Research Associate in the group, and she is developing novel stapled peptides to inhibit medicinally relevant protein–protein interactions.



Daniel H. O'Donovan

Daniel Hillebrand O' Donovan is currently Associate Principal Scientist in Early Oncology R&D at AstraZeneca, Cambridge, UK. Following PhD studies in medicinal chemistry at Trinity College Dublin, he moved to Germany for postdoctoral studies at the Max Planck Institute for Kohlenforschung followed by a Marie Curie fellowship at the University of Oxford, UK. Since joining AstraZeneca in 2016, he has worked in a variety of research areas including antihormonal therapies for breast cancer, epigenetics, immuno-oncology and targeted protein degradation.

Maria Ölwegård Halvarsson

Dr Maria Ölwegård-Halvarsson is a Senior Research Scientist in the New Modalities group, Department of Medicinal Chemistry, AstraZeneca, Sweden. In her current role she develops linker chemistry and synthesizes drug conjugates within the targeting delivery platform. She obtained her PhD in organic chemistry from Gothenburg University in 1990 and then spent four years at the Department of Medicinal Biochemistry, Gothenburg University. She joined AstraZeneca in 1995, working three years in the isotope labelling group and has a 25 year commitment to the company, synthesizing drug molecules within the cardiovascular and metabolic disease areas in lead optimization and lead generation phase.

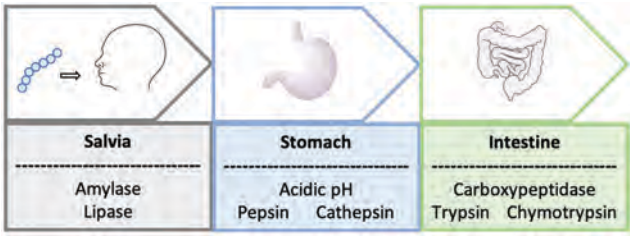


Fig. 1 A schematic to represent the journey of an orally administrated peptide through the gastrointestinal tract.

Santos *et al.* analysed peptides approved by the FDA between 2012–2016 to allow comparison to the Ro5.[4] They determined that the most orally available peptides were indeed up to a MW of 1200 Da and displayed a log *P* within the range of 5–8. Close interpretation of the results found that orally available peptides had 5 times more H-bond donors and acceptors than what was considered as acceptable by Lipinski's Ro5 for small molecules.[4]

Oral administration is challenging for both biologics and peptides and in many cases, it may not be feasible. The whole journey of oral administration through the gastrointestinal tract is problematic, starting with the enzymes amylase and lipase found within saliva that break down the peptides into smaller molecules (Fig. 1).[5] On arrival into the stomach, the peptide is subjected to harsh acidic conditions and proteolysis by cathepsin and pepsin. Even if the peptide successfully remains intact to this point, the lumen of the small intestine experiences a pH change and has a vast number of proteolyzing enzymes including trypsin, chymotrypsin and carboxypeptidase.[5]

Compared to biologics, peptides have a much shorter circulatory half-life (days *vs.* weeks) resulting in the need for sub-optimal frequent drug administrations. The impact of this is witnessed through the advancement of ADCs and a slower progression of PDCs. The lifetime of a hydrophilic peptide in circulation is determined by many soluble enzymes in the blood and at membranes. Exopeptidases are a class of enzymes that can be split into two subgroups: amino- and carboxypeptidases and target the N- and C-terminal, respectively.[6] It is these enzymes that are responsible for the chemical instability and the breakdown of peptides in the bloodstream.

Short half-lives are also experienced due to rapid renal clearance, resulting in many hampered peptide *in vivo* studies and ultimately the pursuit of the peptide as a drug. Found within the kidney are glomeruli pores that have a size of ~8 nm; circulating peptides that are less than 25 kDa filter through the glomeruli, and are not reabsorbed through the renal tubule.[3] Considering such limitations, it is not surprising that many oral peptide drug candidates have entered clinical trials, but with limited success and overall are restricted to the area of endocrine disorders. Formulations are helpful in this setting and have been successfully used in the development of Semaglutide, the most recently FDA approved orally available peptide drug used to treat Type 2 diabetes (September 2019). Sodium *N*-[8-(2-hydroxybenzoyl)amino caprylate] (SNAC) is used with Semaglutide to form a co-formulation.[7] SNAC works as a buffering agent within the stomach, which in turn diminishes the activity of proteolyzing enzymes including pepsin where the maximum activity is experienced at a pH within the range of 2–4.[7] Despite Semaglutide's approval, the delivery of peptides orally still has a long way to develop before we see more in the clinic.

Several ways can be used to try and improve the ADME properties of peptides such as the improvement of the cell permeability, enhancement of chemical and proteolysis stability and reduction of renal clearance overall resulting in the extension of the circulatory half-life. The extended half-life is beneficial both economically and for the patients' compliance. The next part of the review will focus on the ways of modifying a peptide to achieve such improvements.

Improving enzymatic and chemical stability of peptides by chemical modifications

Cyclisation

Cyclisation techniques have been used widely in the peptide field and achieved in several ways from cyclising head to tail, head/tail to side chain or side chain to side chain.

A type of side chain to side chain cyclisation is called stapling, a technique that enables the peptide to be locked into a desired conformation. Peptide stapling is used commonly to enhance a peptide's secondary structure such as α-helices and β-turns, which can improve the binding affinity to the target and enhance ADME properties.

There are two subgroups of peptide stapling (PS): one-component (1C) and two-component (2C). In one-component peptide stapling (1C-PS) there is an intramolecular linkage between often unnatural amino acid side chains and can allow cyclisation depending on the secondary structure.

One of the first examples of 1C-PS was by Blackwell and Grubbs through the use of ring-closing metathesis (RCM) of *O*-allyl serine residues.[8] 1C-PS is not without its limitations. For example, modifications would result in the entire peptide being

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resynthesized, which could prove costly in both time and materials. However, 1C-PS has proved successful in many cases exemplified by the first stapled peptide ALRN-5281 to reach clinical trials and complete Phase I.[9] ALRN-5281 is used to treat adult growth hormone deficiency and was cyclised through the use of 1C-PS *via* RCM.[9]

Peptide stapling more recently has moved the focus away from 1C-PS to two-component peptide stapling (2C-PS) (Fig. 2). The use of 2C-PS offers many advantages over 1C-PS and allows synthetic versatility through the use of linear peptides and separate staples. 2C-PS allows modifications to be made at a late stage if needed to the peptide or the staple, which is highly beneficial for an optimization campaign. 2C-PS has been widely applied to bicycles therapeutics developed by Christian Heinis and Sir Greg Winter.[10] The overall concept of bicycles is the cross-linking of three cysteine residues to a tri-functionalised linker to form a bicycle construct. More details on the application of bicycles for use within drug conjugates are detailed later in the review.

Moving away from proteinogenic amino acids

Side chains of amino acids offer another excellent source for modification with many papers being published recently on direct amino acid modification. Increasing the steric bulk of the side chains results in increased stability, as enzyme recognition is disrupted.[6]

One way to increase the overall stability of the full peptide is to swap L-amino acids to D-amino acids. The D-amino acid sequence has a decreased substrate recognition and binding affinity for proteolytic enzymes.[3] An example of increasing the half-life of a biologically active peptide is modifying Somatostatin to Octreotide used to treat gastrointestinal tumours.[3,6] Octreotide's amino acid sequence incorporates two D-amino acids, whereas Somatostatin is only composed of L-amino acids (Fig. 2). The resulting half-life increases from a few minutes for Somatostatin to 1.5 hours for Octreotide hence enhancing favourable PK properties.[11] Despite this example highlighting the benefits of L- to D-amino acid exchange, there are a few cases in which D-amino acid-containing peptides show a reduced half-life in comparison to the L-analogue.[3] It is important to consider the effects that such modifications could have on the overall secondary structure of the peptide and on any intra- or intermolecular interactions.[3] An alternative is the use of D-peptides. These peptides are mirror images of the L-amino acid-containing counterpart and are composed fully of D-amino acids. The Kay group have recently developed D-peptides for use as HIV entry inhibitors.[12]

Slowing down renal clearance by chemical modifications

The overall net charge for the peptide sequence is an important consideration for renal clearance. Peptides that acquire a net negative charge tend to exhibit a longer half-life in comparison to those with a net positive charge.[13] The presence of anionic carbohydrate moieties found within the kidney's glomerular membrane limit the filtration of anionically charged species into the urine.[13]

Another approach is the conjugation of peptides with larger molecules (>50 kDa) to increase lipophilicity and their binding

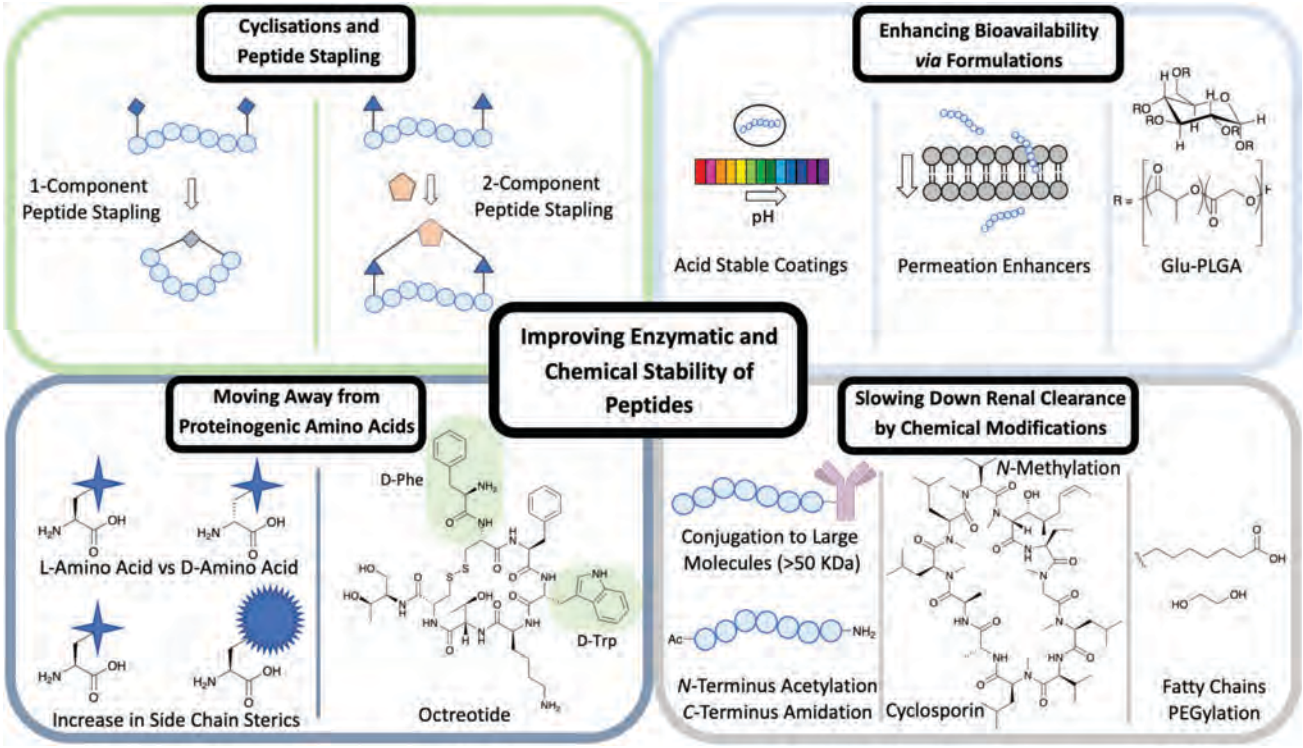

Fig. 2 Different methods used to improve the enzymatic and chemical stability of peptides including: cyclisations and peptide stapling, formulations, moving away from proteinogenic amino acids and chemical modifications.

to albumin, thereby improving the PK and PD properties of peptides.[13] It is the enhanced steric that prevent the conjugate from being filtered out through the kidneys and allow a longer circulation period. Modifications at the N- and C-terminus help slow down renal clearance. Typically the breakdown by exopeptidases of a peptide sequence occurs at the N- or C-terminus.[6]

There are ways this can be prevented by modifying the terminus to increase proteolysis resistance. An amide bond between the C- and N-terminus achieved through a cyclisation reaction has been shown to prevent enzyme degradation.[6] If cyclisation is not the preferred structure for binding, then *N*-acetylation and *C*-amidation can be an alternative to enhance resistance to proteolysis. These modifications have proven successful in a range of studies on Somatostatin, where the half-life of the modified molecule was extended when compared to the unmodified peptide.[3]

N-Methylation of amide bonds is another modification that enhances metabolic resistance. *N*-Methylation increases the steric hindrance and allows tuning of the peptide conformation.[14] Cyclosporin is an example of a naturally occurring peptide used as an immunosuppressive drug (Fig. 2). Cyclosporin is a hepta-*N*-methylated cyclic undecapeptide, with an oral bioavailability of 29%.[14]

The use of polyethylene glycol (PEG) has been vastly explored in slowing down renal clearance and by increasing the binding to plasma proteins such as albumins. PEG is an ideal candidate for modification: it is cheap, biocompatible, hydrophilic, non-toxic and non-immunogenic.[6] The promise of PEGylation for the modification of peptides is highlighted by a number of examples, which are discussed herein.

RGD is a homing tripeptide (see later), whose sequence is able to allow the HM-3 peptide to selectively bind to specific target sites that display high levels of integrins within tumours.[13,15] The original HM-3 peptide had limited effect as a consequence of its short half-life and required twice a day administration. There was a need to enhance the peptide's half-life to reduce the number of administrations needed. Methoxy-poly(ethylene glycol)-aldehyde (mPEG-ald) was the PEG linker of choice for attachment at the N-terminus. Upon this modification, there was a 5.86 fold increase in half-life in male rat studies when compared to the unmodified HM-3 peptide.[15]

Another PEGylated peptide is PEG-adrenomedullin (PEG-ADM) by Bayer used in patients suffering from Acute Respiratory Distress Syndrome (ARDS) associated with lung failure.[16] The peptide was enrolled into Phase 2 clinical trials in August 2020 with the predicted end date of early 2023.[16]

PEG is not the only molecule used in conjugation to the peptide approaches to slow down renal clearance. Other widely used examples include polysialic acids (PSA), a homopolymer, and hydroxyethyl starch (HES), a branched amylopectin.[13]

The addition of fatty chains has been an effective method as an addition to peptides to increase the half-life. Glucagon-like peptide (GLP-1) receptor agonists have been used to control blood sugar levels in patients with Type 2 diabetes.[17] One of the earliest example is Exenatide, an analogue of a nonhuman peptide that in 2005 had twice-daily administration and an IV half-life of 30 minutes. In 2009 Liraglutide, a near analogue of human GLP-1, had a fatty acid chain with a spacer joined to the main peptide backbone for binding to albumin (Fig. 3). Liraglutide represented a significant improvement of GLP-1 with an extended IV half-life of 8–10 hours and once-daily administration.[17] Semaglutide is a GLP-1 agonist, with a γGlu-2xOEG linker to a C_{18} fatty chain (Fig. 3).[17] The determined IV half-life was 46.1 hours through studies involving mini pigs, enabling once-weekly doses – a vast improvement to the early GLP-1 agonists.[17]

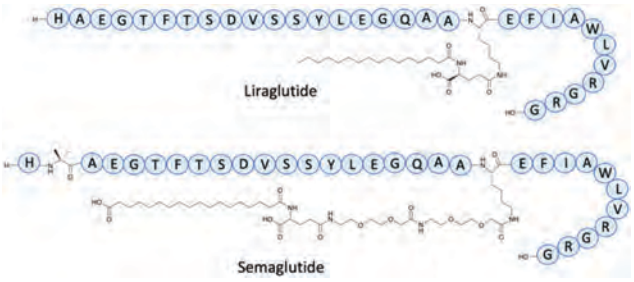

Fig. 3 The structures of Liraglutide and Semaglutide.

Enhancing bioavailability *via* formulations

Several methods have been used previously in the literature to enhance the oral bioavailability of peptide therapeutics *via* formulations.[5] These can include permeation enhancers and acid-stable coatings (Fig. 2).[5] Permeation enhancers are able to transport the peptide through epithelial cells – an alternative route is available through intercellular junction and adhesion protein interference resulting in a paracellular route.[5] The use of acid-stable coatings to improve the oral availability of a drug is a widely used approach. These coatings are pH active, where at low pH in the stomach the coating remains intact; as the peptide moves to the intestine the pH rises and the coating breaks down to release the contents. The introduction of citric acid can be used to help neutralise the optimum basic pH conditions for a range of gastrointestinal peptidases, hence slowing down degradation caused by peptidases.[5]

Formulations can be used to enhance the bioavailability of IV administrated peptides. The FDA approved Sandostatin LAR is an excellent example of this in which Octreotide is encapsulated in a glucose-poly(lactide-*co*-glycolide) (Glu-PLGA) star-shaped polymer (Fig. 2).[18]

Improving the overall chemical and enzymatic stability of a peptide using the techniques discussed is beneficial for the discovery of new therapeutics including targeted therapies exemplified by peptide–drug conjugates. These concepts will be discussed in the following section.

Cancer therapy and targeted drug delivery

Cancer is among the leading contributors to human mortality and disease, with nearly 50% of people being diagnosed with

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