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In vivo degradation forms, anti-degradation strategies, and clinical applications of therapeutic peptides in non-infectious chronic diseases

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Abstract

Current medicinal treatments for diseases comprise largely of two categories: small molecule (chemical) drugs (*e.g.*, aspirin) and larger molecules (peptides/proteins, *e.g.*, insulin). Whilst both types of therapeutics can effectively treat different diseases, ranging from well-understood (in view of pathogenesis and treatment) examples (*e.g.*, flu), to less-understood chronic diseases (*e.g.*, diabetes), classical small molecule drugs often possess significant side-effects (a major cause of drug withdrawal from market) due to their low- or non-specific targeting. By contrast, therapeutic peptides, which comprise short sequences from naturally occurring peptides/proteins, commonly demonstrate high target specificity, well-characterized modes-of-action, and low or non-toxicity *in vivo*. Unfortunately, due to their small size, linear permutation and lack of tertiary structure, peptidic drugs are easily subject to rapid degradation or loss *in vivo* through chemical and physical routines, thus resulting in a short half-life and reduced therapeutic efficacy, a major drawback that can reduce therapeutic efficiency. However, recent studies demonstrate that the short half-life of peptidic drugs can be significantly extended by various means, including use of enantiomeric or non-natural amino acids (AAs) (*e.g.*, L-AAs replacement with D-AAs), chemical conjugation [*e.g.*, with polyethylene glycol], and encapsulation (*e.g.*, in exosomes). In this context, we provide an overview of the major *in vivo* degradation forms of small therapeutic peptides in the plasma and anti-degradation strategies. We also update on the progress of small peptide therapeutics that are either currently in clinical trials or are being successfully used in clinical therapies for patients with non-infectious diseases, such as diabetes, multiple sclerosis, and cancer.

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Author contributions

YT: original manuscript draft; JQH: conceptualization and revision; RGG: reviewing and editing.

Declaration of competing interest.

RGG holds stock in and is a company officer at the Tiny Cargo Company Inc., which has licensed exosomal technology from Virginia Tech. RGG is also a non-remunerated scientific advisory board member and stockholder of FirstString Research Inc, which licensed αCT1 peptide from the Medical University of South Carolina.

Keywords

therapeutic peptide; *in vivo* degradation forms; anti-degradation strategies; clinical applications

1. Introduction

Whilst most common diseases pose no risk to life and manifest only temporary symptoms, a significant fraction of individuals suffer chronic and life-altering pathologies (Vos et al., 2015). For example, diabetes is one of the most prevalent lifelong diseases, affecting 26.9 million adult Americans in 2018, with 1.5 million new cases appearing every year [Centers for Disease Control and Prevention, CDC (CDC, 2020)]. In 2017, the total costs ascribed to diabetes reached $327 billion in the USA (Petersen, 2018). Over 90–95% of these cases are type 2 diabetes (also known as insulin-resistant diabetes), which is traditionally managed by limiting ingestion of carbohydrates, introducing exercise into daily routine, and taking anti-diabetic medications to reduce blood glucose levels (Chatterjee et al., 2017). Although relatively effective in mitigating disease, symptom management does not represent a permanent cure (Genovese et al., 2017). On the other hand, type 1 diabetes (also known as “juvenile diabetes” or “insulin-dependent diabetes”) afflicts over 1.4 million Americans (CDC, 2020). Peptidic insulin peptide is the most well-developed treatment for type 1 diabetes (Atkinson et al., 2014); however, it still poses a risk of inconvenience and economic burden for most patients, as insulin has to be injected daily to control blood glucose levels.

Autoimmune disorders represent another example of major, life-altering diseases - producing a significant economic, physical, and emotional burden to millions of people worldwide (Kirsch-Volders et al., 2020). Multiple sclerosis (MS) (Picard et al., 2015) is one of the more common auto-immune disorders afflicting over 2.3 million people worldwide and over 1 million adults in the USA (McGinley et al., 2021; Wallin et al., 2019). Current treatment is to slow down disease progression and manage symptoms with corticosteroids, physical therapy, and other medications (McGinley et al., 2021); however, no curative treatment is available for MS.

Cardiovascular disease (CVDs) is the number one cause of death worldwide, affecting over 100 million people in the US annually. In 2018 alone, 121.5 million Americans were living with one form of cardiovascular disease or another (Benjamin et al., 2019). Each year, 1.5 million Americans suffer from heart attacks and strokes, accounting for $320 billion in healthcare costs and lost productivity (Giedrimiene and King, 2017). By 2030, it is expected that the costs for CVDs will rise to $818 billion (Giedrimiene and King, 2017). Unfortunately, treatments available for CVDs are mainly to prevent or delay disease progression before it becomes life threatening and no permanent curative options are accessible for treatment of CVD (Aguilar-Ballester et al., 2021; Wintrich et al., 2020).

Small therapeutic peptides are emerging as promising therapy avenues for chronic diseases, with potential to address shortcomings of current standard of care (Lau and Dunn, 2018) (also see Fig. 1). Traditionally, therapeutics can be classified into two groups based on their molecular weights: a). Small chemical molecules [<500 dalton (Da)] that are typically given via oral administration, and b) Peptide and protein-based biologics (>5,000 Da) that

are usually administrated intravenously. However, in recent years, a large number of new peptidic therapeutics with a wide range of molecular sizes from 500 to 5,000 Da have also been reported (Craik et al., 2013). These peptides are usually simple linear amino acids (AAs) chains with some secondary, but no tertiary structures (Marqus et al., 2017; Sato et al., 2006). Typically, therapeutic peptides are designed and synthesized as mimetics of amino acid (AA) sequences found in naturally occurring polypeptides that correspond to specific binding sites for enzymes, receptors and other protein domains. These sequences often show highly target specificity and tend to have reduced potential for immunogenicity if sufficiently short (<6 AAs) (Marqus et al., 2017; McGregor, 2008). Even if a specific functional sequence is not known, peptides with sequence overlapping the putative target site can be readily designed and produced for further screening to identify peptides with optimized targeting affinity (Marqus et al., 2017). By specifically and efficiently disrupting protein-protein interactions, peptides have emerged as promising candidate molecules for clinical therapy (Lau and Dunn, 2018; McGregor, 2008).

In 2015, there were over 140 therapeutic peptides under clinical trials and with over 500 in preclinical studies (Fosgerau and Hoffmann, 2015). Therapeutic peptides have been used in clinic to treat various diseases, including type 1 and 2 diabetes, cancer, and MS (Craik et al., 2013; Genovese et al., 2017; Han and Youn, 2019). For example, Exenatide (*i.e.*, Bydureon® or Byetta®) [a glucagon-like-peptide-1 (GLP-1) agonist] (Genovese et al., 2017) and lixisenatide (*i.e.*, Adlyxin® or Lyxumia®) [an analogue of the human GLP-1] (Nauck et al., 2021; Trujillo et al., 2021) are currently used for patients with type 2 diabetes; while Abarelix (*i.e.*, Plenaxis®) [a gonadotropin-releasing hormone antagonist composed of 10 synthetic AAs that inhibit testosterone production in males] is applied to prostate cancer patients (Garnick and Mottet, 2012; Moul, 2014). Interestingly, Bortezomib (*i.e.*, Velcade®) peptide, which contains only 2 AAs, is the first proteasome inhibitor that has been approved for treatment of multiple myeloma and mantle cell lymphoma (Chen et al., 2011). In the field of cardiovascular diseases, Nesiritide (*i.e.*, Natrecor®) [a recombinant 32-AA peptide of human B-type natriuretic peptide]) is probably the first peptide used for patients with heart failure (Elkayam et al., 2002) (also see Table 2).

The above being said, short therapeutic peptides also come with drawbacks (Fig. 1), including susceptibility to breakdown by degradative enzymes such as endogenous proteases and phosphatases *in vivo* (McGregor, 2008). Even though peptides can be chemically modified to include stable building blocks, several chemical reactions that occur *in vivo* are still able to rapidly degrade peptides: hydrolysis, deamidation, isomerization, diketopiperazine formation, oxidation and disulfide exchange being among such reactions (Furman et al., 2015; Xu et al., 2017). Furthermore, when introduced into the circulatory system, small peptides are subject to renal clearance and thus rapidly cleared from the bloodstream through the glomeruli with minimal retention (Di, 2015; Wu and Huang, 2018). Via chemical degradation and physical elimination, only a small percentage of peptides typically remains bioavailable following intravenous injection, and thus the level of peptidic drug administrated may not be high enough to be effective against the disease process targeted.

Regardless of these barriers, small therapeutic peptides have demonstrated promise in basic research studies using animals and in the human clinical trials. In this review, we provide an overview of the major forms of *in vivo* degradation of small therapeutic peptides in the plasma, and anti-degradation strategies. We also address the progress of small therapeutic peptides that are either undergoing clinical testing, or have been successfully used as clinical therapies, for patients with non-infectious diseases, such as diabetes, multiple sclerosis, and cancer. Whilst therapeutic peptides based on viral sequences have also been tested as treatments for infectious diseases, including human immunodeficiency virus (HIV)-induced acquired immunodeficiency (AIDS) and hepatitis, these are not addressed in this review. For readers interested in this area the following references provide further information (Caillat et al., 2020; McKinnell and Saag, 2009; Skwarecki et al., 2021).

2. Half-life and therapeutic efficacy of small therapeutic peptides

Half-life (*i.e.*, elimination half-life) in pharmacokinetics refers to the time elapsed for the concentration of a given substance to decrease to half of its starting concentration in the plasma or blood (Toutain and Bousquet-Melou, 2004); while the therapeutic efficacy denotes the ability of a given drug to produce desired beneficial effects (Adkins and Noble, 1998). Both are critical parameters and often used in basic research and clinical studies, as well as by the pharmaceutical industry to determine dosage and dosage intervals to achieve efficacy, whilst avoiding potential toxicity and unwanted accumulation of agent in human organs and tissues (Grover and Benet, 2011; Sahin and Benet, 2008). Generally speaking, for a drug with a short half-life, a maximum therapeutic efficiency may not be reached even with frequent administration; while for a drug with a very long half-life, undesired accumulation of the drug in the body may occur and lead to toxicity (Smith et al., 2018).

In the case of therapeutic peptides, most of them have half-lives of only a few minutes due to rapid *in vivo* degradation, renal clearance, and low (membrane) permeability (Werle and Bernkop-Schnurch, 2006). Chronic diseases, such as cancer, cardiovascular disorders, and MS noted above, typically need a long or lifelong therapy duration. In such contexts, peptides with very short half-lives tend not to be beneficial, showing reduced potential for efficacy over longer treatment regimens (Marqus et al., 2017). In addition, peptides normally have low oral bioavailability (*i.e.*, unsuitable for oral administration) owing to acid hydrolysis and digestive enzymes in the stomach, as well as showing low absorption in the gastrointestinal track (Bruno et al., 2013; Lau and Dunn, 2018). Although other routes, such as intravenous or subcutaneous injection, may overcome delivery issues (Craig et al., 2018), the short half-lives of most peptides in the plasma and other body fluids as a result of multiple degradation forms, also places limits on the potential for therapeutic benefit. To this end, successful development of new methods that can increase half-life of therapeutic peptides *in vivo* to levels comparable to small molecule drugs will be critical for making peptidic drugs more effective and thus promoting their widespread use as clinical treatments.

3. *In vivo* forms of chemical degradation of small therapeutic peptides

Chemically-associated degradations are denoted as alternation of peptide structure and/or its molecular composition, which in turn lead to changes in peptide properties (Furman et al.,

2015). Seven primary types of chemically-associated degradation have been found to alter peptide structure, each of which are directly or indirectly associated with shorter half-life *in vivo*, peptide degradation, and reduction of the final concentration of peptide. These include: 1) disulfide bond formation, 2) hydrolysis, 3) diketopiperazine formation, 4) isomerization, 5) deamidation, 6) oxidation, and 7) proteases/phosphatase enzyme-associated catalytic degradations (Furman et al., 2015; Vickers et al., 2002; Xu et al., 2017) (Fig. 2).

3.1. Disulfide bond formation

The formation of disulfide bond is known to regulate multiple biological functions. It not only stabilizes 3 dimensional (3D) structure of proteins, but also participates in enzymatic catalysis, protects proteins from oxidative damage, promotes protein aggregation, and reduces target binding affinity (Chandrasekhar et al., 2014; Chiu and Hogg, 2019), acting like a “double-edged sword” in modulating the stability of peptide and proteins. Although disulfide bond formation is mainly associated with enhanced stability of peptides and proteins (Yeo and Rabenstein, 1993), molecular modification by disulfide bond formation can also lead to changes in chemical and physical properties, leading to decreased solubility (and concentration) of peptides and proteins because of aggregation induced by disulfide bonds (Chandrasekhar et al., 2014; Chiu and Hogg, 2019). For instance, double cystine peptides (2 AAs) connected by a disulfide bond have much lower solubilities than those with a single cystine in the human plasma (Bannai, 1984). In a case where the solubility of a peptide containing cystine is required for therapeutic purposes, cystine connection by disulfide bonds can reduce the solubility of peptide in the plasma. Interestingly, in large proteins, disulfide bonds are critical for stabilization of 3D structure; whilst in small peptides of less than 50 AAs, they appear to have less structural significance (Chiu and Hogg, 2019; Cook and Hogg, 2013).

3.2. Hydrolysis

Hydrolysis is defined as a chemical cleavage involving addition of water molecules (Korhonen et al., 2006). The plasma is mainly composed of water; thus, hydrolysis is one of the major reactions in the circulatory system that contributes to peptide degradation (Powell et al., 1993). In addition, the plasma contains various types of enzymes, such as proteases and phosphatases, which are directly responsible for enzymatically-associated peptide degradation (see 3.7 Proteases and phosphatases-associated degradation section below). For example, human angiotensin-converting enzyme-related carboxypeptidase (ACE2) can break down biologically active peptides by removing their carboxyl-terminus (C-terminus), through catalyzing hydrolysis between proline and a hydrophobic AA (Vickers et al., 2002). In another example, asparagine residues in peptide chains can catalyze hydrolysis reactions at C-terminus of a peptide by acting as a proton donor and attacking the peptide bond between asparagine and other AAs under acidic conditions (Furman et al., 2015).

3.3. Diketopiperazine formation

Diketopiperazine is a 6-member ring structure linked by 2 amides (Mieczkowski et al., 2021). It originates from the amino-terminus (N-terminus or NH_2-terminus) residues of a peptide chain and introduces issues for synthesis and storage of peptides (Capasso et al., 1998). The reaction occurs when the nitrogen atom of the N-terminal deprotonated amino

group attacks the carbonyl carbon atom of the second AA residue, resulting in breakdown of the polypeptide chain and formation of a ring (Capasso et al., 1998). The significant change in chemical structure can alter stability, biological compatibility, and target affinity of peptides (Marsden et al., 1993). It should be noted that all reported studies regarding diketopiperazine formation appeared to be conducted in bacteria and in test tubes. Although its formation in mammalian cells, or in the plasma remains unknown, gut microbiota-derived diketopiperazine (Correa et al., 2019) may directly or indirectly affect the stability of plasma peptides once it is absorbed in the blood stream?

3.4. Isomerization

Isomerization refers to transformation of a molecule into its optical or geometric isoform (Morgenstern, 2009). It occurs through chemical reactions, such as deamidation (Robinson, 2002). Additionally, the isomerization enzymes in mammalian cells can catalyze such reactions and thus, are capable of altering structure and function of proteins and peptides (Zhang et al., 2021). In the case of proteins and peptides, it takes place by altering secondary structure; while in AAs, all naturally occurring AAs exist in two isomeric forms, *i.e.*, dextrorotatory (D) and levorotatory (L), with the exception of glycine (Young et al., 1994). Proteins and peptides in the human body are all composed of L-AAs; whereas D-AAs are not typically found in naturally occurring proteins or peptides, and consequently sequences comprising these enantiomeric AAs tend to be resistant to endogenous degradation mechanisms (Young et al., 1994), thus isomerization can also be used as an anti-degradation strategy (see 5.1.2 Chemical Isomerization section below). Although this type of anti-degradation approach is beneficial, the resulting peptides with D-AAs usually come with altered stability, solubility, permeability, and binding affinities than those with L-AAs, which can lead to decreases in activity and thus efficacy of the therapeutic peptide (Baker et al., 1993). For instance, Acyclovir, an antiviral drug used in the treatment of herpes simplex virus-1 (HSV-1) induced corneal keratitis, has a higher corneal permeability in L-aspartate form than in D-aspartate form of Acyclovir (Majumdar et al., 2009).

3.5. Deamidation

Deamidation is a chemical reaction in which the amide functional group of AAs is removed through non-enzymatic or enzymatic processes (Stamnaes et al., 2008; Wright, 1991). AAs, such as glutamine and asparagine, are the main residues that are subjected to non-enzymatic deamidation *in vivo* (Furman et al., 2015; Robinson, 2002; Xu et al., 2017). The deamidation rate of AAs are determined by various properties, including neighboring AAs, 3D structure, potential hydrogen (pH), temperature, and buffer ionic strength (Robinson, 2002). For example, at physiological pH, deamidation generates negatively charged residues that can affect the properties (such as binding affinity) of proteins in biologically significant ways (Robinson, 2002). Asparagine deamidation, for example, is probably one of the most common deamidations that is associated with protein degradation (Yang and Zubarev, 2010). Deamidation of asparagine occurs through a five membered succinimide ring intermediate that can form various products, including L-isoasparagine, D-isoasparagine, and D-asparagine (Yang and Zubarev, 2010). Introducing different conformations or isoforms of asparagine that can alter binding affinity, solubility, and permeability; thus,

affecting half-life and therapeutic efficacy. As described above, deamidation can also result in isomerization, which in turn can change the properties of therapeutic peptides.

3.6. Oxidation

Oxidation-reduction reactions occur in all cells and are critical for homeostasis, cell signaling, gene expression, energy metabolism, aerobic respiration, cell growth, and apoptosis (Zhao et al., 2014). A molecule is oxidized when it loses an electron due to an oxidative agent (Whayne et al., 2016). An oxidative agent is usually another molecule that can be reduced by taking up electrons lost by the oxidized species (Koppenol and Hider, 2019; Whayne et al., 2016). For instance, L-methionine oxidation results in formation of L-methionine sulfoxide and L-methionine sulfone, both of which have different structures - with additional oxygen atoms binding to the sulfur atom than in L-methionine (Furman et al., 2015). In addition, certain oxidation-reduction reactions can also lead to the formation of reactive oxygen species (ROS). ROS can be both beneficial (as intracellular messengers, biological activity modulators) (Mitra et al., 2019) and harmful [by damaging proteins, lipids, and deoxyribonucleic acid (DNA) through oxidation reactions] (Mrakic-Sposta et al., 2014). Even though ROS activity mainly takes place intracellularly, reactions at the cell membrane can release ROS into the extracellular matrix and bloodstream, where these highly reactive species can act on, and alter the structure polypeptides in the external milieu (Barelli et al., 2008; Mrakic-Sposta et al., 2014). Chemical oxidation of proline, threonine, histidine, arginine and/or lysine produces carbonyl derivatives of peptide/proteins (Butterfield et al., 1998; Phaniendra et al., 2015); while carbonylation of peptides/proteins is an irreversible process that often leads to loss of function of the oxidized molecules (Akagawa, 2021).

3.7. Proteases and phosphatases-associated degradation

Another major category of chemically-associated *in vivo* degradation is catalyzed by proteases and phosphatases, two major enzymes related with peptide degradation in the plasma. These enzymes are significantly responsible for hydrolysis of peptide bonds as exemplified in Fig. 2 [also see (Vickers et al., 2002)]. The major functions of the plasma enzymes involve regulation of protein activities and protein-protein interactions, as well as participation in intercellular and intracellular signaling via breakdown of proteins and peptide chains (Lopez-Otin and Bond, 2008).

Proteases are traditionally classified into two groups based on their action sites: a) Endogenous proteases that act on internal peptide bonds; and b) Exogenous proteases that act, near or on, the C- or N-termini of peptide chains or proteins (Bond, 2019; Lopez-Otin and Bond, 2008). Endogenous proteases can be further divided into subcategories according to the AA residue that they act upon, such as aspartic, cysteine, glutamic, metallo, serine, and threonine proteases (Bond, 2019). Aspartic, glutamic, and metalloproteases act on the peptide bond by using a water molecule as an electron donor while cysteine, serine and threonine proteases cleave peptide bonds by using an AA residue (cysteine, serine or threonine) as an electron donor (Lopez-Otin and Bond, 2008). Glutamic proteases are not found in mammals; however, their mechanism of action is believed to be similar to aspartic and metalloproteases occurring in humans (Lopez-Otin and Bond, 2008).

Up to date, over 550 proteases and protease homologues have been identified in the human body (Puente et al., 2003), which also include those only discovered in the gastrointestinal tract and tissues, as well as others found in the blood plasma (Bottger et al., 2017). For example, the plasma thrombin and factor Xa are two key proteases that regulate blood coagulation (Posma et al., 2019); while Kallikrein is a prominent plasma protease that selectively cleaves arginine- and lysine-bonds of proteins and peptides (Kishibe, 2019). Another important type of plasma protease is a complement molecule in the complement system, which play essential roles in the innate immune response (Sim and Tsiftsoglou, 2004). The complement system is composed of different types of enzymes that interact with one another to promote opsonization and the inflammatory response (Dunkelberger and Song, 2010; Lu and Kishore, 2017; Tomlinson and Thurman, 2018). In the intestines and intestinal lumen, aminopeptidases (P, W, N) and dipeptidyl peptidase-IV, chymotrypsin, trypsin, and carboxypeptidases are examples of many known key proteases (Gentilucci et al., 2010). Protease inhibitor-based anti-degradation approaches may lead to the development of new methods to protect therapeutic peptide or enhance their half-lives (De Leuw and Stephan, 2018; Festa et al., 2021).

Protein phosphatases are broadly categorized into two main groups, *i.e.*, serine/threonine phosphatases and tyrosine phosphatases, based on the AA residues from which they remove the phosphate group (Otsubo et al., 2018). Phosphorylation of phosphatases by kinases trigger catalytical reactions resulting in the removal of phosphate groups, which in turn results in either activation or inhibition of the targeted protein and the downstream cascades within which it participates (Reddy et al., 2017; Swingle and Honkanen, 2019). Such activation or inhibition can lead to processes such as protease enzyme activation, which promotes peptide degradation. For instance, protein phosphatase 1 is a serine/threonine phosphatase that is known to remove a phosphate group from retinoblastoma susceptibility (Rb) protein in humans (Morana et al., 1996), while Rb kinase plays an opposite role, adding a phosphate group to the Rb protein. Understanding the dual effects of phosphatase and kinases on therapeutic peptides/proteins may promote clinical applications of existing and future therapeutic biologics (Morana et al., 1996).

3.8. Cytochrome P450 (CYP or P450)-associated drug degradation

CYP is a superfamily member of hemeproteins that are critical in natural (chemical substances produced by living organisms) and synthetic (chemical substances synthesized in laboratory or pharmaceutical industry) drug metabolism (McDonnell and Dang, 2013; Tornio and Backman, 2018). In humans, there are 18 gene families and 44 gene subfamilies encoding numerous CYP enzymes (Tornio and Backman, 2018). Almost all CYP gene subfamilies are expressed in the liver, where the majority of the drug metabolism occurs, while some members of the CYP subfamilies are also expressed in other organs, such as brain, lungs, and intestines (McDonnell and Dang, 2013; Tornio and Backman, 2018).

Each CYP subfamily protein (enzyme) is known to play a different role in drug metabolism. For example, the CYP2C subfamily (accounting for 20–25% in the hepatic CYP contents) metabolize about 15–20% of all drugs [*e.g.*, nonsteroidal anti-inflammatory drugs (NSAIDs), hypoglycemics, anticonvulsant drugs, and angiotensin II blockers] (Isvoran

et al., 2017; Tornio and Backman, 2018); while the CYP2D6 subfamilies are responsible for metabolism of more than 160 drugs (or about 25% of currently prescribed drugs, like antidepressants, beta-blockers, and opioids) (He et al., 2015; Rutman et al., 2021). For the metabolism of endogenous compounds such as cholesterol, bile-acid, steroids, fatty acids, and vitamin D, other CYP families (*e.g.*, CYP7, CYP17, CYP27) seem to be involved (Chiang and Ferrell, 2020; Nebert and Russell, 2002). Although their critical roles in metabolizing a wide-range drugs are well characterized, the CYP superfamily appear not to contribute to the metabolism of biological agents (Serra Lopez-Matencio et al., 2018). Whether this is also the case for small therapeutic peptides remains unknown.

4. Physical elimination and molecular incompatibility of small therapeutic peptides

Physical elimination (*i.e.*, physical clearance) refers to bodily activities that simply eliminate (*e.g.*, filtered through glomerulus in the kidney) peptides from the bloodstream without altering the peptide's physical or chemical properties (Good et al., 2010). Renal clearance from the circulatory system is a major elimination route to reduce drug concentration in the plasma, thus decreasing the half-life and therapeutic efficacy of drugs (including peptides) that should be considered if and when therapeutic peptides come to be more widely used in clinical settings (Zaman et al., 2019). The kidney glomeruli have a pore size of ~8 nm, so peptides with molecular weights of <25 kDa are readily filtered from blood; and unfortunately, peptides that pass through the glomeruli can't be absorbed through the renal tubules, which further aggravate losses of bioavailable peptide (Di, 2015; Wu and Huang, 2018). Since small therapeutic peptides have molecular weight <5 kDa (Craik et al., 2013), they are readily filtered from the blood via the glomeruli.

Another major physical factor that reduces therapeutic efficiency of therapeutic peptides is molecular incompatibility between peptides and cell membranes (Dunican and Doherty, 2001). The cell membrane is composed of a lipid bilayer that is almost impermeable to all charged (polar and nonpolar) molecules and uncharged (polar) molecules (Yang and Hinner, 2015). Mammalian cell membrane has active transporters that utilize energy to transport compounds across the membrane, such as ion channels, and passive transporters that do not require energy, such as aquaporins (Yang and Hinner, 2015). Unfortunately, transporters or channels in the cell membrane that are specific for conveying small therapeutic peptides have not been reported. Because most therapeutic peptides are hydrophilic (polar), and thus have limited ability to pass through the hydrophobic lipid bilayer composing biological membranes (Teixeira et al., 2012), they do not readily pass into the cytoplasm [many peptidic drugs have intracellular targets (Jones and Sayers, 2012)] through passive diffusion. As a result, peptide not taken up by cells has susceptibility to degradation by eliminative routes or clearance by the kidney (Zaman et al., 2019).

5. Anti-degradation strategies used in small therapeutic peptides

5.1 Current methods

A significant amount of work has been conducted with the intention of increasing the *in vivo* stability and half-life of small therapeutic peptides in animal models and human patients. Four approaches that are commonly used in the field are summarized below (also see Table 1):

5.1.1. Modification of C- or N-terminus—In this approach, the C- or/and N-terminus of peptide chain is biochemically modified, thus preventing interactions with proteases (Di, 2015). Exogenous proteases typically affect peptide or protein by binding to their C- or N-terminus and promote reactions that cleave peptide bonds (Lopez-Otin and Bond, 2008). Modifications such as acetylation or glycosylation of the N-terminus or amidation of the C-terminus can prevent binding of exogenous proteases, protecting the peptide from enzymatic degradation (Sato et al., 2006).

In other instances, acetylation of the Werner (WRN) protein is known to increase its stability, whereas acetylation of the p53 C-terminus decreases ubiquitination, extending half-life *in vivo* (Li et al., 2002). Additionally, amidation of human GLP-1 enhances stability and half-life in plasma (Wettergren et al., 1998). *In vivo* studies demonstrating the effectiveness of exogenous N- and C- terminus modifications in preventing degradation have also been applied to peptide-based therapies. For example, N-terminal acetylation of GLP-1 was found to protect from degradation by dipeptidyl peptidase-IV (DPP-IV), increasing half-life from minutes to up to two hours (John et al., 2008).

5.1.2. Chemical isomerization—D-AAs do not exist naturally *in vivo* and most plasma proteases cannot degrade peptide chains that contain one or more D-AAs (Di, 2015); thus, chemical replacement of L-AAs with D-AAs prevents or delays peptide degradation (Domhan et al., 2019). It is known that D-AAs are structural stereoisomers of natural AAs (*i.e.*, L-AAs) and alteration of peptide stereoisomerism can negatively affect protease binding and affinity (Gentilucci et al., 2010). Peptides composed of D-AAs have been shown to be subjected to less enzymatic degradation *in vivo* (Asano, 2012; Gentilucci et al., 2010). Desmopressin is probably the most striking example of how chemical isomerization increases peptide half-life, wherein replacement of L-arginine with D-arginine increased half-life from 10 min to 3.7 hrs (Agerso et al., 2004; Di, 2015; Wisniewski et al., 2019).

5.1.3. Molecular conjugation—Chemical conjugation of a peptide with another macromolecule can provide significant protective effects against degradation (Tartibi et al., 2016). Polyethylene glycol (PEG) is a polyether that has high water solubility, low toxicity, and high mobility in solution (Knop et al., 2010; Sato et al., 2006). PEG is a large molecule, on which peptides can be attached via their C- or N-terminus. Because of its large size, PEG has a low renal clearance rate; thus, conjugation prevents or decreases conjugated peptides from being filtered out by the kidneys (Pasut and Veronese, 2009). Furthermore, being attached by peptide termini, PEG-conjugation provides protection from proteolytic activities of enzymes that act on C- or N-termini (Caliceti and Veronese, 2003; Knop et al., 2010).

Peptides can be also conjugated with the plasma proteins, such as albumin, a monomeric protein that has a long half-life (Bumbaca et al., 2019). Due to its chemical composition and large size, albumin has a low renal clearance, thus prolonging the half-life of the conjugated peptide (Bumbaca et al., 2019; Sato et al., 2006). In humans, albumin has a half-life of ~21 days because it is protected from catabolic elimination by neonatal fragment crystallizable (Fc) receptor-mediated recycling (Muller et al., 2007; Roopenian et al., 2015). Being an abundant native protein with a long half-life and limited immunogenicity or toxicity (Roopenian et al., 2015), albumin has become a useful molecular tool for stabilizing therapeutic peptides (Low and Wiles, 2016). As an example, the albumin-conjugated therapeutic peptide, Albiglutide (Tanzeum®), is a Food and Drug Administration (FDA, USA) approved-drug for treatment of type 2 diabetes (Bronden et al., 2017; Poole and Nowlan, 2014). This conjugated peptide has a half-life of 6 to 7 days, which not only significantly decreases frequency of drug administration, but also maintains normal blood levels of glucose up to a week after each injection (Sato et al., 2006).

5.1.4. Drug delivery devices—Drug delivery devices, which are often manufactured from nonbiodegradable materials (*e.g.*, alloys), provide another strategy for protecting and delivering small therapeutic peptides (Nahar et al., 2006). For example, DUROS® is a tube-shaped drug delivery device that is made from a nonbiodegradable titanium alloy containing a drug reservoir chamber and an orifice from which the peptide is released (Rohloff et al., 2008). This device uses osmotic pressure through an osmotic engine to slowly release a predetermined amount of peptide into the body, thus making the device capable of administering therapeutics for 3 to 12 months, depending on the properties of the therapeutic molecules (Rohloff et al., 2008). In the case of leuprolide (also known as leuprorelin) (Lupron®), a peptide for patients with prostate cancer, the device can continuously administer peptide for up to 12 months - with results that appear to show clinical promise (Crawford et al., 2015; Fowler et al., 2000; Rohloff et al., 2008).

The insulin pump is a well-known example of a successful drug delivery device that is used by diabetic patients worldwide. It was first built in the 1970s with the goal of providing a better quality of life for patients with type 1 diabetes (Didangelos and Iliadis, 2011). Although there are multiple brands of insulin pumps on the market, the general components are similar - comprising an insulin reservoir capable of holding sufficient insulin for a few days, a catheter that is inserted subcutaneously for insulin delivery, a battery, and a processor that adjusts the frequency and amount of insulin delivered (Didangelos and Iliadis, 2011).

5.2 Emerging tactics

Whilst the methods listed above are presently used in the clinic, new approaches for controlled delivery of peptide therapeutics are also attracting interest (also see Table 1):

5.2.1 Nanoparticles—Nanoparticles, which can be made from the organic (*e.g.*, dendrimers, polymersomes) or inorganic (*e.g.*, gold, silica) materials, refer to particles with a size range between 1–100 nm in diameter (Mirza and Siddiqui, 2014). Due to their nanoscale size, high ratio of surface area to volume, high bioavailability and high stability, nanoparticles are a promising transport platform for facilitating drug delivery (Mahmoudian

et al., 2021; Singh and Lillard, 2009). Following intravenous injection, nanoparticles can freely circulate in the blood, appear to readily penetrate tissues, and be taken up by cells (Patra et al., 2018). When drugs (*e.g.*, small molecular chemical compounds, peptides, or even proteins), especially those with low absorption and poor solubility, are conjugated with/within nanoparticles, it has been hypothesized that drug delivery efficiency and efficacy in targeted tissues/cells can be dramatically increased (Kalimuthu et al., 2018; Patra et al., 2018).

To generate nanoparticles, peptides are usually dissolved in an appropriate solvent and drug is then entrapped, absorbed, or attached to the nanoparticle. Nanoparticles with different properties can also be engineered to have different solubilities, binding affinities, peptide release ratios, and desired mechanisms of action, including protection against degradative enzymes such as proteases (Singh and Lillard, 2009). By modifying their surface chemistry, or construction from biodegradable polymers, nanoparticles can increase drug solubility in the plasma or provide sustained release to target tissues over controlled and/or extended periods (Singh and Lillard, 2009).

Due to high levels of biocompatibility, tolerance of pH variance and low toxicity gold nanoparticles are a preferred drug delivery vehicle (Sani et al., 2021), examples of which have been “green-lighted” by the FDA for clinical studies in humans (Dhar et al., 2008; Mirza and Siddiqui, 2014). Gold nanoparticles can be synthesized in different sizes and coated with different materials for specific purposes that accord with the molecule to which they are conjugated (Rana et al., 2012; Ronavari et al., 2021). As an example, PEG-coated gold nanoparticles were demonstrated to be able to carry peptides or even proteins (Kalimuthu et al., 2018; Rana et al., 2012).

5.2.2 Exosomes—Extracellular vesicles (EVs) are lipid bilayer-bound vesicles that are naturally secreted by almost all types of cells into the extracellular space (Doyle and Wang, 2019). Based on their size, cellular origin, and morphology, EVs can be classified into 3 groups, apoptotic bodies (1–5 μm, blebbing of apoptotic cells, irregular), microvesicles (100–1000 nm, plasma membrane shedding of living cells, irregular), and exosomes (30–100 nm, endosome of living cells, regular) (Todorova et al., 2017).

Exosomes have attracted particular attention as they are released from living cells via exocytosis and contain proteins, lipids, messenger ribonucleic acids (mRNAs), microRNAs (miRNAs), as well as specific markers/receptors [*e.g.*, cluster of differentiation 31 (CD31), also known as platelet endothelial cell adhesion molecule-1 (PECAM-1), for endothelial cells] of cellular origin, which are thought to enable exosomes to recognize and target cells, doing so whilst not prompting the immune system (Yuan et al., 2017). Once bound to their target, exosomes fuse with the cell membrane, deploying signaling cargo directly into the cytoplasm and triggering intracellular signaling pathways (Bunggulawa et al., 2018). Furthermore, exosomes have been posited to travel through the bloodstream without being subject to unduly rapid degradation or renal clearance prior to uptake in target tissues and organs (Saeedi et al., 2019). Since exosomes are mainly secreted into the circulation, they can be readily harvested for encapsulating drugs of interest (*e.g.*, small therapeutic peptides) before being delivered to diseased tissues and cells (Bunggulawa et al., 2018). Exosomes

can also be isolated in abundance from other bodily fluids including milk (Marsh et al., 2021a). Because of these properties, exosomes have demonstrated superior abilities not only to protect drugs from degradation, but also to increase drug delivery efficacy and specificity to the target cells (Doyle and Wang, 2019).

Overall, by using approaches discussed above, individually or in combination, the short half-lives of small therapeutic peptides could be able to be extended in a clinically significant manner. Chronic diseases such as cancer, cardiovascular disorders, and auto-immune disorders, thus may be more efficiently treated through mechanisms by directly targeting intracellular signaling pathways via naturally occurring biological molecules (*e.g.*, protein, mRNA, miRNA).

6. Examples of major small therapeutic peptides that are currently used in clinical therapy for major non-infection diseases

To date, around 360 clinical trials have tested therapeutic peptides (https://clinicaltrials.gov/ct2/home; keywords: “therapeutic peptides”). The following sections provide examples of several therapeutic peptidic drugs that are being used in the clinic or may soon advance to this point (also see Table 2).

6.1. Insulin (i.e., Humulin R®)

Insulin is the first, most well-known, and fully developed therapeutic peptide that has been routinely used in the clinic, and at home, to treat patients with diabetes (Jain et al., 2020; Kurtzhals et al., 2021). Insulin, a 51-AA peptide, was first discovered in 1910, but its initial clinical testing was not carried out until 1922 (Karamitsos, 2011). Prior to 1978, therapeutic insulin could be only isolated from animals’ (*e.g.*, pigs and cows) pancreatic glands (Johansen, 1983). Although animal-sourced insulins have been used for many decades in patients with diabetes, the major drawbacks of these insulins (even highly purified) comprise immune reactions and short time of activity following administration in the human body (Schernthaner, 1993).

Up until 1978, the first human insulin was successfully produced in *Escherichia coli (E. coli)* by Genentech (San Francisco, CA) (Goeddel et al., 1979) and 4 years later, this biosynthetic type of insulin was made available commercially (Johansen, 1983). Meanwhile, another type of semisynthetic human insulin, which was derived from animal insulin via enzymatic substitution of AA, was also effectively used in clinic to treat diabetic patients (Quatraro et al., 1989; Raptis and Dimitriadis, 1985). These significant achievements paved the way for mass-production of variants of insulin and insulin delivery devices (pumps) for treating diabetes at different levels of severity (Hirsch et al., 2020; Nathan, 2015). The advance included rapid-acting, short-acting, intermediate-acting, long-acting, and ultra-long acting insulins, as well as mixtures of these, depending on patient need (Hirsch et al., 2020; Kjeldsen et al., 2021). The administration frequency of pump-based insulin delivery, and the amount of insulin, can also be easily adjusted in the personalized treatment of diabetes (Nathan, 2015). These synthetic molecules represent a real achievement in human

health, signifying a case in point of the outstanding advantages and clinical benefits over the original methods used to isolate insulin (King et al., 2016).

The breakthrough of successful insulin therapy also triggered researchers to seek new peptides in combating other diseases, such as genetic disorders that have deficiencies of certain enzymes or proteins (Sheikh and Yokota, 2021). In the past decades, multiple novel therapeutic peptides for type 2 diabetes, myeloma, breast and prostate cancer, MS, and cardiovascular diseases have been successfully applied in clinic, as described below.

6.2. Exenatide (i.e., Byetta®) and lixisenatide (i.e., Adlyxin®)

Exenatide, a 39-AA peptide, is the first GLP-1 agonist approved by the FDA for treating type 2 diabetes (Genovese et al., 2017). It is administered intravenously as a suspension containing the peptide encapsulated in microspheres, which allows for slow release of the peptide (Lim et al., 2015). In the human plasma, it has an approximately 2.4-hour half-life following release (Hall et al., 2018). With twice-a-day administration, Exenatide significantly increased the life quality and productivity of patients with type 2 diabetes (McCormack, 2014). Lixisenatide is an analogue of human GLP-1, which is also used in type 2 diabetes therapy (Baker and Levien, 2017; Meier, 2012)}. This 44-AA peptide functions through binding to and activating the GLP-1 receptor, resulting in secretion of GLP-1 and prompting pancreatic beta cells to release insulin in response to elevated levels of blood glucose (Nauck et al., 2021; Trujillo et al., 2021). Because lixisenatide C-terminus is amidated, with an amide group instead of a carboxyl group, the peptide is protected against degradation, especially by the dipeptidyl peptidase-4 enzyme (Meier, 2012; Werner et al., 2010).

6.3. Glatiramer acetate (i.e., Copaxone®)

One of the most widely used small therapeutic peptides is glatiramer acetate, a 10-AA peptide for treatment of MS (Craik et al., 2013; Scotto et al., 2021). This peptidic drug was designed as a synthetic analogue of myelin basic protein, the major protein associated with MS (Wynn, 2019). Although the underlying mechanism of drug action is not completely understood, the FDA approved the peptide in 1996 (Wynn, 2019). Clinical data from the European, American, and Canadian trials indicate that Copaxone® is an effective therapeutic that can decrease the frequency of MS relapse (Comi et al., 2001; Ford et al., 2010). Copaxone is injected subcutaneously along with the inactive ingredient mannitol that acts as a vehicle (Corominas et al., 2014; Rieckmann et al., 2021).

6.4. Leuprolide (also known as leuprorelin, i.e., Lupron®)

Leuprolide, a 9-AA peptide, is a synthetically modified version of gonadotropin-releasing hormone and used for treatment of breast and prostate cancer, as well as central precocious puberty (Adjei et al., 1990; Bereket, 2017; Han and Youn, 2019; Mejia-Otero et al., 2021; Salazar et al., 2021). Clinical data indicates that prolonged treatment with this peptide significantly desensitizes gonadotropin-releasing hormone receptors and decreases plasma levels of testosterone in males and estradiol in females, resulting in inhibition of hormone-dependent tumor growth in breast and prostate cancers (Han and Youn, 2019; Snelder et al., 2019; Swayzer and Gerriets, 2021). Like exenatide, leuprolide has a short half-life (~3

hours), which makes the drug impractical for continuous administration to patients. As a result, leuprolide is encapsulated in biodegradable lipophilic synthetic microspheres, which allows slow release between 1 to 4 months, dependent on the polymer type used during microsphere synthesis (Periti et al., 2002). An alternative method using a nonbiodegradable implant can also sustain slow release of leuprolide (Wright, 2010).

6.5. Abarelix (i.e., Plenaxis®)

Abarelix is a gonadotropin-releasing hormone antagonist composed of 10 AAs, 5 of which are non-natural that prevent the peptide from rapid degradation (Mongiat-Artus and Teillac, 2004). Abarelix is produced in powder form and then dissolved in sodium chloride to make as a depot solution to allow for controlled release following intramuscular injection (Mongiat-Artus and Teillac, 2004). Mechanistically, abarelix functions by competitively binding to gonadotropin-releasing hormone receptors, inhibiting expression of luteinizing hormone and follicle stimulating hormone and thereby preventing production of testosterone in males, thus slowing or inhibiting the growth of prostate cancer (Nauck et al., 2021; Trujillo et al., 2021). Unfortunately, clinical use of abarelix was discontinued in the US due to allergic reaction, though it remains in use in Germany and the Netherlands (Moul, 2014).

6.6. Bortezomib (i.e., Velcade®)

Bortezomib is a striking example of 2-AA proteasome inhibitor. It was the first peptide-based proteasome inhibitor approved by the FDA for treating patients with newly diagnosed and relapsed multiple myeloma and mantle cell lymphoma (Chen et al., 2011). Bortezomib is an N-protected (pyrazinoic acid) dipeptide boronic acid derivative (Laforgia et al., 2021). It is composed of pyrazinoic acid, L-phenylalanine and L-leucine that have boronic acid in its carboxylic acid group (Pyz-Phe-boroLeu) (Chen et al., 2011). By binding to the 26S proteasome reversibly through its boronic acid group and inhibiting its function, bortezomib activates intracellular signaling pathways that leads to death of lymphoma cells (Chen et al., 2011; Romancik et al., 2022). Although bortezomib is not exclusively constructed from AAs, the peptide backbone-based structure has attracted researchers to focus on development of other small peptide-like therapeutics that could have efficacy in the treatment of cancer (Mahmoudian et al., 2021).

6.7. Nesiritide (i.e., Natrecor®)

Nesiritide, a 32-AA peptide, is a recombinant version of human B-type natriuretic peptide (Elkayam et al., 2002). It is used for patients with decompressed heart failure, in which the heart loses its ability to provide adequate amounts of blood and oxygen to the body (Mangini et al., 2013). Nesitritide is administered intravenously in hospital settings and acts as a vasodilator to increase the cardiac output in patients with heart failure (Gottlieb et al., 2013). Although its half-life in the plasma is approximately 18 min (Hobbs and Mills, 1999), direct intravenous administration allows the peptide concentration to be maintained in an adequate range for desired therapeutic effects (Gottlieb et al., 2013).

6.8. Alpha-carboxyl terminus 1 peptide (αCT1)(i.e., Granexin®)

αCT1 is a 25-AA peptide that is currently in pivotal-stage clinical trials for surgical scar reduction and skin radiation injury (DiCarlo et al., 2020; Montgomery et al., 2021). This 25mer comprises a 16-AA antennapedia cell penetration sequence and the last 9 AAs of the gap junction protein connexin 43 (Hunter et al., 2005). In Phase II clinical trials, αCT1 was also shown to have beneficial effects in the healing of chronic skin wounds, including diabetic foot ulcers and venous leg ulcers (Ghatnekar et al., 2015; Ghatnekar et al., 2009). αCT1 is one of a few peptides that is delivered topically to skin instead of intravenously, which makes it an interesting example of an alternative delivery approach for therapeutics of this type (Montgomery et al., 2018). Formulations of αCT1 tested pre-clinically have included incorporation into alginate microcapsules (Moore et al., 2013) and poly-lactic-co-glycolic acid (PLGA) nanoparticles designed to enable controlled sustained release in disease settings (Roberts et al., 2020).

Overall, therapeutic peptides can be manufactured as a slightly altered version of natural molecules or exactly mimic sequences endogenous to the body. This unique property (naturally derived) offers great therapeutic benefits with high target specificity, high bioavailability, and potential low toxicity compared to conventional small molecule drugs (Sato et al., 2006). In sum, successful clinical testing and application of therapeutic peptides could promote the wider-spread application of these large drug-like molecules in the treatment of a range of human pathologies.

7. Conclusion

Small therapeutic peptides are typically linear sequences of less than 50 AAs. As such molecules are often synthesized to correspond to native endogenous peptides, they inherently tend to exhibit low toxicity, together with high target specificity and biocompatibility. Whilst there have been significant advances in preclinical development, many small peptide-based therapies face obstacles – most especially related to their instability and short half-life once introduced into the body. Due to their small size, lack of tertiary structure and hydrophilicity, small peptides are particularly prone to chemical (enzymatic) degradation and physical elimination (renal clearance), which leads to reductions in therapeutic efficiency. Fortunately, techniques such as peptide conjugation with nanoparticles or macromolecules (*e.g.*, PEG), C- or N-terminal modification, and replacement of L-AAs with D-AAs have shown promise to address these issues. A novel and recent innovation is to protect peptidic drugs, and other fragile biological molecules (*e.g.*, miRNAs) by encapsulation in exosome-based drug delivery vehicles. As a consequence of new technologies such as exosomal delivery, small therapeutic peptides stand at a threshold that may see their increased usage for a variety of unmet clinical needs – most especially as urgently needed medications for serious and chronic diseases, including cancer, autoimmune, and cardiovascular diseases.

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References

Adjei A, Doyle R, Pratt M, Finley R, Johnson E, 1990. Bioavailability of leuprolide following intratracheal administration to beagle dogs. Int. J. Pharm 61, 135–144. 10.1016/0378-5173(90)90052-6.

Adkins JC, Noble S, 1998. Tiagabine. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in the management of epilepsy. Drugs 55, 437–460. 10.2165/00003495-199855030-00013. [PubMed: 9530548]

Agerso H, Seiding Larsen L, Riis A, Lovgren U, Karlsson MO, Senderovitz T, 2004. Pharmacokinetics and renal excretion of desmopressin after intravenous administration to healthy subjects and renally impaired patients. Br. J. Clin. Pharmacol 58, 352–358. 10.1111/j.1365-2125.2004.02175.x. [PubMed: 15373927]

Aguilar-Ballester M, Hurtado-Genoves G, Taberner-Cortes A, Herrero-Cervera A, Martinez-Hervas S, Gonzalez-Navarro H, 2021. Therapies for the treatment of cardiovascular disease associated with type 2 diabetes and dyslipidemia. Int. J. Mol. Sci 22, 660–687. 10.3390/ijms22020660. [PubMed: 33440821]

Akagawa M, 2021. Protein carbonylation: Molecular mechanisms, biological implications, and analytical approaches. Free. Radic. Res 55, 307–320. 10.1080/10715762.2020.1851027. [PubMed: 33183115]

Asano Y, 2012. Enzymes acting on d-amino acid containing peptides. Methods Mol. Biol 794, 397–406. 10.1007/978-1-61779-331-8_27. [PubMed: 21956579]

Atkinson MA, Eisenbarth GS, Michels AW, 2014. Type 1 diabetes. Lancet 383, 69–82. 10.1016/S0140-6736(13)60591-7. [PubMed: 23890997]

Baker DE, Levien TL, 2017. Lixisenatide. Hosp. Pharm 52, 65–80. 10.1310/hpj5201-65. [PubMed: 28179743]

Baker MA, Maloy WL, Zasloff M, Jacob LS, 1993. Anticancer efficacy of magainin2 and analogue peptides. Cancer Res. 53, 3052–3057. https://www.ncbi.nlm.nih.gov/pubmed/8319212. [PubMed: 8319212]

Bannai S, 1984. Transport of cystine and cysteine in mammalian cells. Biochim. Biophys. Acta 779, 289–306. 10.1016/0304-4157(84)90014-5. [PubMed: 6383474]

Barelli S, Canellini G, Thadikkaran L, Crettaz D, Quadroni M, Rossier JS, Tissot JD, Lion N, 2008. Oxidation of proteins: Basic principles and perspectives for blood proteomics. Proteomics Clin. Appl 2, 142–157. 10.1002/prca.200780009. [PubMed: 21136821]

Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN, Djousse L, Elkind MSV, Ferguson JF, Fornage M, Jordan LC, Khan SS, Kissela BM, Knutson KL, Kwan TW, Lackland DT, Lewis TT, Lichtman JH, Longenecker CT, Loop MS, Lutsey PL, Martin SS, Matsushita K, Moran AE, Mussolino ME, O'Flaherty M, Pandey A, Perak AM, Rosamond WD, Roth GA, Sampson UKA, Satou GM, Schroeder EB, Shah SH, Spartano NL, Stokes A, Tirschwell DL, Tsao CW, Turakhia MP, VanWagner LB, Wilkins JT, Wong SS, Virani SS, American Heart Association Council on, E., Prevention Statistics, C., Stroke Statistics, S., 2019. Heart disease and stroke statistics-2019 update: A report from the american heart association. Circulation 139, e56–e528. 10.1161/CIR.0000000000000659. [PubMed: 30700139]

Bereket A, 2017. A critical appraisal of the effect of gonadotropin-releasing hormon analog treatment on adult height of girls with central precocious puberty. J. Clin. Res. Pediatr. Endocrinol 9, 33–48. 10.4274/jcrpe.2017.S004. [PubMed: 29280737]

Bond JS, 2019. Proteases: History, discovery, and roles in health and disease. J. Biol. Chem 294, 1643–1651. 10.1074/jbc.TM118.004156. [PubMed: 30710012]

Bottger R, Hoffmann R, Knappe D, 2017. Differential stability of therapeutic peptides with different proteolytic cleavage sites in blood, plasma and serum. PLoS One 12, e0178943. 10.1371/journal.pone.0178943. [PubMed: 28575099]

Bronden A, Knop FK, Christensen MB, 2017. Clinical pharmacokinetics and pharmacodynamics of albiglutide. Clin. Pharmacokinet 56, 719–731. 10.1007/s40262-016-0499-8. [PubMed: 28050889]

Bruno BJ, Miller GD, Lim CS, 2013. Basics and recent advances in peptide and protein drug delivery. Ther. Deliv 4, 1443–1467. 10.4155/tde.13.104. [PubMed: 24228993]

Bumbaca B, Li Z, Shah DK, 2019. Pharmacokinetics of protein and peptide conjugates. Drug. Metab. Pharmacokinet 34, 42–54. 10.1016/j.dmpk.2018.11.001. [PubMed: 30573392]

Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, Wang G, 2018. Recent advancements in the use of exosomes as drug delivery systems. J. Nanobiotechnology 16, 81–94. 10.1186/s12951-018-0403-9. [PubMed: 30326899]

Butterfield DA, Koppal T, Howard B, Subramaniam R, Hall N, Hensley K, Yatin S, Allen K, Aksenov M, Aksenova M, Carney J, 1998. Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants n-tert-butyl-alpha-phenylnitrone and vitamin e. Ann. N. Y. Acad. Sci 854, 448–462. 10.1111/j.1749-6632.1998.tb09924.x. [PubMed: 9928452]

Caillat C, Guilligay D, Sulbaran G, Weissenhorn W, 2020. Neutralizing antibodies targeting hiv-1 gp41. Viruses 12, 1210-. 10.3390/v12111210. [PubMed: 33114242]

Caliceti P, Veronese FM, 2003. Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. Adv. Drug. Del. Rev 55, 1261–1277. 10.1016/s0169-409x(03)00108-x.

Capasso S, Vergara A, Mazzarella L, 1998. Mechanism of 2,5-dioxopiperazine formation. J. Am. Chem. Soc 120, 1990–1995. 10.1021/ja972051a.

CDC. National diabetes statistics report - estimates of diabetes and its burden in the united states. 2020:1–21. https://https://www.cdc.gov/diabetes/data/statistics-report/index.html.

Chandrasekhar S, Epling DE, Sophocleous AM, Topp EM, 2014. Thiol-disulfide exchange in peptides derived from human growth hormone. J. Pharm. Sci 103, 1032–1042. 10.1002/jps.23906. [PubMed: 24549831]

Chatterjee S, Khunti K, Davies MJ, 2017. Type 2 diabetes. Lancet 389, 2239–2251. 10.1016/S0140-6736(17)30058-2. [PubMed: 28190580]

Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP, 2011. Bortezomib as the first proteasome inhibitor anticancer drug: Current status and future perspectives. Curr. Cancer Drug Targets 11, 239–253. 10.2174/156800911794519752. [PubMed: 21247388]

Chiang JYL, Ferrell JM, 2020. Up to date on cholesterol 7 alpha-hydroxylase (cyp7a1) in bile acid synthesis. Liver Res. 4, 47–63. 10.1016/j.livres.2020.05.001. [PubMed: 34290896]

Chiu J, Hogg PJ, 2019. Allosteric disulfides: Sophisticated molecular structures enabling flexible protein regulation. J. Biol. Chem 294, 2949–2960. 10.1074/jbc.REV118.005604. [PubMed: 30635401]

Comi G, Filippi M, Wolinsky JS, 2001. European/canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging--measured disease activity and burden in patients with relapsing multiple sclerosis. European/canadian glatiramer acetate study group. Ann. Neurol 49, 290–297. https://www.ncbi.nlm.nih.gov/pubmed/11261502. [PubMed: 11261502]

Cook KM, Hogg PJ, 2013. Post-translational control of protein function by disulfide bond cleavage. Antioxid. Redox Signal 18, 1987–2015. 10.1089/ars.2012.4807. [PubMed: 23198756]

Corominas M, Postigo I, Cardona V, Lleonart R, Romero-Pinel L, Martinez J, 2014. Ige-mediated allergic reactions after the first administration of glatiramer acetate in patients with multiple sclerosis. Int. Arch. Allergy Immunol 165, 244–246. 10.1159/000371418. [PubMed: 25634237]

Correa Y, Cabanillas B, Jullian V, Alvarez D, Castillo D, Dufloer C, Bustamante B, Roncal E, Neyra E, Sheen P, Sauvain M, 2019. Identification and characterization of compounds from chrysosporium multifidum, a fungus with moderate antimicrobial activity isolated from hermetia illucens gut microbiota. PLoS One 14, e0218837. 10.1371/journal.pone.0218837. [PubMed: 31860650]

Craig CM, Liu LF, Nguyen T, Price C, Bingham J, McLaughlin TL, 2018. Efficacy and pharmacokinetics of subcutaneous exendin (9–39) in patients with post-bariatric hypoglycaemia. Diabetes Obes. Metab 20, 352–361. 10.1111/dom.13078. [PubMed: 28776922]

Craik DJ, Fairlie DP, Liras S, Price D, 2013. The future of peptide-based drugs. Chem. Biol. Drug Des 81, 136–147. 10.1111/cbdd.12055. [PubMed: 23253135]

Crawford ED, Moul JW, Sartor O, Shore ND, 2015. Extended release, 6-month formulations of leuprolide acetate for the treatment of advanced prostate cancer: Achieving testosterone levels below 20 ng/dl. Expert Opin. Drug Metab. Toxicol 11, 1465–1474. 10.1517/17425255.2015.1073711. [PubMed: 26293510]

De Leuw P, Stephan C, 2018. Protease inhibitor therapy for hepatitis c virus-infection. Expert Opin. Pharmacother 19, 577–587. 10.1080/14656566.2018.1454428. [PubMed: 29595065]

Dhar S, Reddy EM, Shiras A, Pokharkar V, Prasad BL, 2008. Natural gum reduced/stabilized gold nanoparticles for drug delivery formulations. Chemistry (Easton) 14, 10244–10250. 10.1002/chem.200801093.

Di L, 2015. Strategic approaches to optimizing peptide adme properties. AAPS J. 17, 134–143. 10.1208/s12248-014-9687-3. [PubMed: 25366889]

DiCarlo AL, Bandremer AC, Hollingsworth BA, Kasim S, Laniyonu A, Todd NF, Wang SJ, Wertheimer ER, Rios CI, 2020. Cutaneous radiation injuries: Models, assessment and treatments. Radiat. Res 194, 315–344. 10.1667/RADE-20-00120.1. [PubMed: 32857831]

Didangelos T, Iliadis F, 2011. Insulin pump therapy in adults. Diabetes Res. Clin. Pract 93 Suppl 1, S109–113. 10.1016/S0168-8227(11)70025-0. [PubMed: 21864741]

Domhan C, Uhl P, Kleist C, Zimmermann S, Umstatter F, Leotta K, Mier W, Wink M, 2019. Replacement of l-amino acids by d-amino acids in the antimicrobial peptide ranalexin and its consequences for antimicrobial activity and biodistribution. Molecules 24, 2987–2999. 10.3390/molecules24162987. [PubMed: 31426494]

Doyle LM, Wang MZ, 2019. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells 8, 727–751. 10.3390/cells8070727. [PubMed: 31311206]

Dunican DJ, Doherty P, 2001. Designing cell-permeant phosphopeptides to modulate intracellular signaling pathways. Biopolymers 60, 45–60. 10.1002/1097-0282(2001)60:1<45::AID-BIP1003>3.0.CO;2-9. [PubMed: 11376432]

Dunkelberger JR, Song WC, 2010. Complement and its role in innate and adaptive immune responses. Cell Res. 20, 34–50. 10.1038/cr.2009.139. [PubMed: 20010915]

Elkayam U, Akhter MW, Tummala P, Khan S, Singh H, 2002. Nesiritide: A new drug for the treatment of decompensated heart failure. J. Cardiovasc. Pharmacol. Ther 7, 181–194. 10.1177/107424840200700308. [PubMed: 12232567]

Festa L, Roth LM, B KJ, Geiger JD, Jordan-Sciutto KL, Grinspan JB, 2021. Protease inhibitors, saquinavir and darunavir, inhibit oligodendrocyte maturation: Implications for lysosomal stress. J. Neuroimmune Pharmacol 16, 169–180. 10.1007/s11481-019-09893-8. [PubMed: 31776836]

Ford C, Goodman AD, Johnson K, Kachuck N, Lindsey JW, Lisak R, Luzzio C, Myers L, Panitch H, Preiningerova J, Pruitt A, Rose J, Rus H, Wolinsky J, 2010. Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: Results from the 15-year analysis of the us prospective open-label study of glatiramer acetate. Mult. Scler 16, 342–350. 10.1177/1352458509358088. [PubMed: 20106943]

Fosgerau K, Hoffmann T, 2015. Peptide therapeutics: Current status and future directions. Drug Discov. Today 20, 122–128. 10.1016/j.drudis.2014.10.003. [PubMed: 25450771]

Fowler JE Jr., Gottesman JE, Reid CF, Andriole GL Jr., Soloway MS, 2000. Safety and efficacy of an implantable leuprolide delivery system in patients with advanced prostate cancer. J. Urol 164, 730–734. 10.1097/00005392-200009010-00026. [PubMed: 10953135]

Furman JL, Chiu M, Hunter MJ, 2015. Early engineering approaches to improve peptide developability and manufacturability. AAPS J. 17, 111–120. 10.1208/s12248-014-9681-9. [PubMed: 25338742]

Garnick MB, Mottet N, 2012. New treatment paradigm for prostate cancer: Abarelix initiation therapy for immediate testosterone suppression followed by a luteinizing hormone-releasing hormone agonist. BJU Int. 110, 499–504. 10.1111/j.1464-410X.2011.10708.x. [PubMed: 22093775]

Genovese S, Mannucci E, Ceriello A, 2017. A review of the long-term efficacy, tolerability, and safety of exenatide once weekly for type 2 diabetes. Adv. Ther 34, 1791–1814. 10.1007/s12325-017-0499-6. [PubMed: 28674957]

Gentilucci L, De Marco R, Cerisoli L, 2010. Chemical modifications designed to improve peptide stability: Incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. Curr. Pharm. Des 16, 3185–3203. 10.2174/138161210793292555. [PubMed: 20687878]

Ghatnekar GS, Grek CL, Armstrong DG, Desai SC, Gourdie RG, 2015. The effect of a connexin43-based peptide on the healing of chronic venous leg ulcers: A multicenter, randomized trial. J. Invest. Dermatol 135, 289–298. 10.1038/jid.2014.318. [PubMed: 25072595]

Ghatnekar GS, O'Quinn MP, Jourdan LJ, Gurjarpadhye AA, Draughn RL, Gourdie RG, 2009. Connexin43 carboxyl-terminal peptides reduce scar progenitor and promote regenerative healing following skin wounding. Regen. Med 4, 205–223. 10.2217/17460751.4.2.205. [PubMed: 19317641]

Giedrimiene D, King R, 2017. Abstract 207: Burden of cardiovascular disease (cvd) on economic cost. Comparison of outcomes in us and europe. Circ. Cardiovasc. Qual. Outcomes 10, A207–A207. 10.1161/circoutcomes.10.suppl_3.207.

Goeddel DV, Kleid DG, Bolivar F, Heyneker HL, Yansura DG, Crea R, Hirose T, Kraszewski A, Itakura K, Riggs AD, 1979. Expression in escherichia coli of chemically synthesized genes for human insulin. Proc. Natl. Acad. Sci. U. S. A 76, 106–110. 10.1073/pnas.76.1.106. [PubMed: 85300]

Good DM, Zurbig P, Argiles A, Bauer HW, Behrens G, Coon JJ, Dakna M, Decramer S, Delles C, Dominiczak AF, Ehrich JH, Eitner F, Fliser D, Frommberger M, Ganser A, Girolami MA, Golovko I, Gwinner W, Haubitz M, Herget-Rosenthal S, Jankowski J, Jahn H, Jerums G, Julian BA, Kellmann M, Kliem V, Kolch W, Krolewski AS, Luppi M, Massy Z, Melter M, Neususs C, Novak J, Peter K, Rossing K, Rupprecht H, Schanstra JP, Schiffer E, Stolzenburg JU, Tarnow L, Theodorescu D, Thongboonkerd V, Vanholder R, Weissinger EM, Mischak H, Schmitt-Kopplin P, 2010. Naturally occurring human urinary peptides for use in diagnosis of chronic kidney disease. Mol. Cell. Proteomics 9, 2424–2437. 10.1074/mcp.M110.001917. [PubMed: 20616184]

Gottlieb SS, Stebbins A, Voors AA, Hasselblad V, Ezekowitz JA, Califf RM, O'Connor CM, Starling RC, Hernandez AF, 2013. Effects of nesiritide and predictors of urine output in acute decompensated heart failure: Results from ascend-hf (acute study of clinical effectiveness of nesiritide and decompensated heart failure). J. Am. Coll. Cardiol 62, 1177–1183. 10.1016/j.jacc.2013.04.073. [PubMed: 23747790]

Grover A, Benet LZ, 2011. Intermittent drug dosing intervals guided by the operational multiple dosing half lives for predictable plasma accumulation and fluctuation. J. Pharmacokinet. Pharmacodyn 38, 369–383. 10.1007/s10928-011-9198-0. [PubMed: 21499748]

Hall S, Isaacs D, Clements JN, 2018. Pharmacokinetics and clinical implications of semaglutide: A new glucagon-like peptide (glp)-1 receptor agonist. Clin. Pharmacokinet 57, 1529–1538. 10.1007/s40262-018-0668-z. [PubMed: 29915923]

Han W, Youn HJ, 2019. Clinical studies investigating the use of leuprorelin in breast cancer patients from asia. Asian Pac. J. Cancer Prev 20, 1475–1479. 10.31557/APJCP.2019.20.5.1475. [PubMed: 31127911]

He ZX, Chen XW, Zhou ZW, Zhou SF, 2015. Impact of physiological, pathological and environmental factors on the expression and activity of human cytochrome p450 2d6 and implications in precision medicine. Drug Metab. Rev 47, 470–519. 10.3109/03602532.2015.1101131. [PubMed: 26574146]

Hirsch IB, Juneja R, Beals JM, Antalis CJ, Wright EE, 2020. The evolution of insulin and how it informs therapy and treatment choices. Endocr. Rev 41, 733–755. 10.1210/endrev/bnaa015. [PubMed: 32396624]

Hobbs RE, Mills RM, 1999. Therapeutic potential of nesiritide (recombinant b-type natriuretic peptide) in the treatment of heart failure. Expert Opin. Investig. Drugs 8, 1063–1072. 10.1517/13543784.8.7.1063.

Hunter AW, Barker RJ, Zhu C, Gourdie RG, 2005. Zonula occludens-1 alters connexin43 gap junction size and organization by influencing channel accretion. Mol. Biol. Cell 16, 5686–5698. 10.1091/mbc.e05-08-0737. [PubMed: 16195341]

Isvoran A, Louet M, Vladoiu DL, Craciun D, Loriot MA, Villoutreix BO, Miteva MA, 2017. Pharmacogenomics of the cytochrome p450 2c family: Impacts of amino acid variations on drug metabolism. Drug Discov. Today 22, 366–376. 10.1016/j.drudis.2016.09.015. [PubMed: 27693711]

Jain SM, Seshadri K, Unnikrishnan AG, Chawla M, Kalra P, Vipin VP, Ravishankar E, Chordia J, Das S, Wasir J, Bandookwala SM, Deka N, Agarwal G, Vijaykumar G, Erande S, 2020. Best practices and tools for titrating basal insulins: Expert opinion from an indian panel via the modified delphi consensus method. Diabetes Ther. 11, 621–632. 10.1007/s13300-020-00770-9. [PubMed: 32009224]

Johansen K, 1983. Human insulin--medical progress? Metabolism 32, 528–532. 10.1016/0026-0495(83)90019-7. [PubMed: 6341766]

John H, Maronde E, Forssmann WG, Meyer M, Adermann K, 2008. N-terminal acetylation protects glucagon-like peptide glp-1-(7–34)-amide from dpp-iv-mediated degradation retaining camp- and insulin-releasing capacity. Eur. J. Med. Res 13, 73–78. https://www.ncbi.nlm.nih.gov/pubmed/18424366. [PubMed: 18424366]

Jones AT, Sayers EJ, 2012. Cell entry of cell penetrating peptides: Tales of tails wagging dogs. J. Control. Release 161, 582–591. 10.1016/j.jconrel.2012.04.003. [PubMed: 22516088]

Kalimuthu K, Lubin BC, Bazylevich A, Gellerman G, Shpilberg O, Luboshits G, Firer MA, 2018. Gold nanoparticles stabilize peptide-drug-conjugates for sustained targeted drug delivery to cancer cells. J. Nanobiotechnology 16, 34–47. 10.1186/s12951-018-0362-1. [PubMed: 29602308]

Karamitsos DT, 2011. The story of insulin discovery. Diabetes Res. Clin. Pract 93 Suppl 1, S2–8. 10.1016/S0168-8227(11)70007-9. [PubMed: 21864746]

Kim YM, Lee SM, Chung HS, 2013. Novel aglp-1 albumin fusion protein as a long-lasting agent for type 2 diabetes. BMB Rep. 46, 606–610. 10.5483/bmbrep.2013.46.12.106. [PubMed: 24195794]

King AB, Kuroda A, Matsuhisa M, Hobbs T, 2016. A review of insulin-dosing formulas for continuous subcutaneous insulin infusion (csii) for adults with type 1 diabetes. Curr. Diab. Rep 16, 83–91. 10.1007/s11892-016-0772-0. [PubMed: 27457238]

Kirsch-Volders M, Bolognesi C, Ceppi M, Bruzzone M, Fenech M, 2020. Micronuclei, inflammation and auto-immune disease. Mutat. Res 786, 108335–108355. 10.1016/j.mrrev.2020.108335.

Kishibe M, 2019. Physiological and pathological roles of kallikrein-related peptidases in the epidermis. J. Dermatol. Sci 95, 50–55. 10.1016/j.jdermsci.2019.06.007. [PubMed: 31279501]

Kjeldsen TB, Hubalek F, Hjorringgaard CU, Tagmose TM, Nishimura E, Stidsen CE, Porsgaard T, Fledelius C, Refsgaard HHF, Gram-Nielsen S, Naver H, Pridal L, Hoeg-Jensen T, Jeppesen CB, Manfe V, Ludvigsen S, Lautrup-Larsen I, Madsen P, 2021. Molecular engineering of insulin icodec, the first acylated insulin analog for once-weekly administration in humans. J. Med. Chem 64, 8942–8950. 10.1021/acs.jmedchem.1c00257. [PubMed: 33944562]

Knop K, Hoogenboom R, Fischer D, Schubert US, 2010. Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives. Angew. Chem. Int. Ed. Engl 49, 6288–6308. 10.1002/anie.200902672. [PubMed: 20648499]

Koppenol WH, Hider RH, 2019. Iron and redox cycling. Do's and don'ts. Free Radic. Biol. Med 133, 3–10. 10.1016/j.freeradbiomed.2018.09.022. [PubMed: 30236787]

Korhonen H, Hakala RA, Helminen AO, Seppala JV, 2006. Synthesis and hydrolysis behaviour of poly(ester anhydrides) from polylactone precursors containing alkenyl moieties. Macromol. Biosci 6, 496–505. 10.1002/mabi.200600060. [PubMed: 16921537]

Kurtzhals P, Nishimura E, Haahr H, Hoeg-Jensen T, Johansson E, Madsen P, Sturis J, Kjeldsen T, 2021. Commemorating insulin's centennial: Engineering insulin pharmacology towards physiology. Trends Pharmacol. Sci 42, 620–639. 10.1016/j.tips.2021.05.005. [PubMed: 34148677]

Laforgia M, Laface C, Calabro C, Ferraiuolo S, Ungaro V, Tricarico D, Gadaleta CD, Nardulli P, Ranieri G, 2021. Peripheral neuropathy under oncologic therapies: A literature review on pathogenetic mechanisms. Int. J. Mol. Sci 22, 1980–2002. 10.3390/ijms22041980. [PubMed: 33671327]

Lau JL, Dunn MK, 2018. Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorg. Med. Chem 26, 2700–2707. 10.1016/j.bmc.2017.06.052. [PubMed: 28720325]

Li M, Luo J, Brooks CL, Gu W, 2002. Acetylation of p53 inhibits its ubiquitination by mdm2. J. Biol. Chem 277, 50607–50611. 10.1074/jbc.C200578200. [PubMed: 12421820]

Lim SM, Eom HN, Jiang HH, Sohn M, Lee KC, 2015. Evaluation of pegylated exendin-4 released from poly (lactic-co-glycolic acid) microspheres for antidiabetic therapy. J. Pharm. Sci 104, 72–80. 10.1002/jps.24238. [PubMed: 25407390]

Lopez-Otin C, Bond JS, 2008. Proteases: Multifunctional enzymes in life and disease. J. Biol. Chem 283, 30433–30437. 10.1074/jbc.R800035200. [PubMed: 18650443]

Low BE, Wiles MV, 2016. A humanized mouse model to study human albumin and albumin conjugates pharmacokinetics. Methods Mol. Biol 1438, 115–122. 10.1007/978-1-4939-3661-8_7.

Lu J, Kishore U, 2017. C1 complex: An adaptable proteolytic module for complement and non-complement functions. Front. Immunol 8, 592–603. 10.3389/fimmu.2017.00592. [PubMed: 28596769]

Mahmoudian M, Valizadeh H, Lobenberg R, Zakeri-Milani P, 2021. Bortezomib-loaded lipidic-nano drug delivery systems; formulation, therapeutic efficacy, and pharmacokinetics. J. Microencapsul 38, 192–202. 10.1080/02652048.2021.1876175. [PubMed: 33530812]

Majumdar S, Hingorani T, Srirangam R, Gadepalli RS, Rimoldi JM, Repka MA, 2009. Transcorneal permeation of l- and d-aspartate ester prodrugs of acyclovir: Delineation of passive diffusion versus transporter involvement. Pharm. Res 26, 1261–1269. 10.1007/s11095-008-9730-0. [PubMed: 18839288]

Mangini S, Pires PV, Braga FG, Bacal F, 2013. Decompensated heart failure. Einstein (Sao Paulo) 11, 383–391. 10.1590/s1679-45082013000300022. [PubMed: 24136770]

Marqus S, Pirogova E, Piva TJ, 2017. Evaluation of the use of therapeutic peptides for cancer treatment. J. Biomed. Sci 24, 21–36. 10.1186/s12929-017-0328-x. [PubMed: 28320393]

Marsden BJ, Nguyen TM, Schiller PW, 1993. Spontaneous degradation via diketopiperazine formation of peptides containing a tetrahydroisoquinoline-3-carboxylic acid residue in the 2-position of the peptide sequence. Int. J. Pept. Protein Res 41, 313–316. 10.1111/j.1399-3011.1993.tb00340.x. [PubMed: 8385079]

Marsh SR, Pridham KJ, Jourdan J, Gourdie RG, 2021a. Novel protocols for scalable production of high quality purified small extracellular vesicles from bovine milk. Nanotheranostics 5, 488–498. 10.7150/ntno.62213. [PubMed: 34367882]

Marsh SR, Williams ZJ, Pridham KJ, Gourdie RG, 2021b. Peptidic connexin43 therapeutics in cardiac reparative medicine. J. Cardiovasc. Dev. Dis 8, 52–71. 10.3390/jcdd8050052. [PubMed: 34063001]

Mathieu C, Ostenson CG, Matthaei S, Reaney M, Krarup T, Guerci B, Kiljanski J, Salaun-Martin C, Sapin H, Theodorakis M, 2013. Using exenatide twice daily or insulin in clinical practice: Results from choice. Diabetes Ther. 4, 285–308. 10.1007/s13300-013-0037-8. [PubMed: 24018835]

McCormack PL, 2014. Exenatide twice daily: A review of its use in the management of patients with type 2 diabetes mellitus. Drugs 74, 325–351. 10.1007/s40265-013-0172-6. [PubMed: 24435322]

McDonnell AM, Dang CH, 2013. Basic review of the cytochrome p450 system. J. Adv. Pract. Oncol 4, 263–268. 10.6004/jadpro.2013.4.4.7. [PubMed: 25032007]

McGinley MP, Goldschmidt CH, Rae-Grant AD, 2021. Diagnosis and treatment of multiple sclerosis: A review. JAMA 325, 765–779. 10.1001/jama.2020.26858. [PubMed: 33620411]

McGregor DP, 2008. Discovering and improving novel peptide therapeutics. Curr. Opin. Pharmacol 8, 616–619. 10.1016/j.coph.2008.06.002. [PubMed: 18602024]

McKinnell JA, Saag MS, 2009. Novel drug classes: Entry inhibitors [enfuvirtide, chemokine (c-c motif) receptor 5 antagonists]. Curr. Opin. HIV AIDS 4, 513–517. 10.1097/COH.0b013e328331d3d0. [PubMed: 20048719]

Meier JJ, 2012. Glp-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. Nat. Rev. Endocrinol 8, 728–742. 10.1038/nrendo.2012.140. [PubMed: 22945360]

Mejia-Otero JD, White P, Lopez X, 2021. Effectiveness of puberty suppression with gonadotropin-releasing hormone agonists in transgender youth. Transgend. Health 6, 31–35. 10.1089/trgh.2020.0007. [PubMed: 33614957]

Mieczkowski A, Speina E, Trzybinski D, Winiewska-Szajewska M, Winska P, Borsuk EM, Podsiadla-Bialoskorska M, Przygodzki T, Drabikowski K, Stanczyk L, Zhukov I, Watala C, Wozniak K, 2021. Diketopiperazine-based, flexible tadalafil analogues: Synthesis, crystal structures and biological activity profile. Molecules 26, 794–816. 10.3390/molecules26040794. [PubMed: 33546456]

Mirza AZ, Siddiqui FA, 2014. Nanomedicine and drug delivery: A mini review. Int. Nano. Lett 4, 94–102. 10.1007/s40089-014-0094-7.

Mitra S, Nguyen LN, Akter M, Park G, Choi EH, Kaushik NK, 2019. Impact of ros generated by chemical, physical, and plasma techniques on cancer attenuation. Cancers (Basel) 11, 1060–1091. 10.3390/cancers11071030. [PubMed: 31357584]

Mongiat-Artus P, Teillac P, 2004. Abarelix: The first gonadotrophin-releasing hormone antagonist for the treatment of prostate cancer. Expert Opin. Pharmacother 5, 2171–2179. 10.1517/14656566.5.10.2171. [PubMed: 15461552]

Montgomery J, Ghatnekar GS, Grek CL, Moyer KE, Gourdie RG, 2018. Connexin 43-based therapeutics for dermal wound healing. Int. J. Mol. Sci 19, 1778–1789. 10.3390/ijms19061778. [PubMed: 29914066]

Montgomery J, Richardson WJ, Marsh S, Rhett JM, Bustos F, Degen K, Ghatnekar GS, Grek CL, Jourdan LJ, Holmes JW, Gourdie RG, 2021. The connexin 43 carboxyl terminal mimetic peptide alphact1 prompts differentiation of a collagen scar matrix in humans resembling unwounded skin. FASEB J. 35, e21762. 10.1096/fj.202001881R. [PubMed: 34246197]

Moore K, Amos J, Davis J, Gourdie R, Potts JD, 2013. Characterization of polymeric microcapsules containing a low molecular weight peptide for controlled release. Microsc. Microanal 19, 213–226. 10.1017/S143192761201389X. [PubMed: 23360728]

Morana SJ, Wolf CM, Li J, Reynolds JE, Brown MK, Eastman A, 1996. The involvement of protein phosphatases in the activation of ice/ced-3 protease, intracellular acidification, DNA digestion, and apoptosis. J. Biol. Chem 271, 18263–18271. 10.1074/jbc.271.30.18263. [PubMed: 8663484]

Morgenstern K, 2009. Isomerization reactions on single adsorbed molecules. Acc. Chem. Res 42, 213–223. 10.1021/ar800021q. [PubMed: 19138111]

Moul JW, 2014. Utility of lhrh antagonists for advanced prostate cancer. Can. J. Urol 21, 22–27. https://www.ncbi.nlm.nih.gov/pubmed/24775720. [PubMed: 24775720]

Mrakic-Sposta S, Gussoni M, Montorsi M, Porcelli S, Vezzoli A, 2014. A quantitative method to monitor reactive oxygen species production by electron paramagnetic resonance in physiological and pathological conditions. Oxid. Med. Cell. Longev 2014, 306179–306190. 10.1155/2014/306179. [PubMed: 25374651]

Muller D, Karle A, Meissburger B, Hofig I, Stork R, Kontermann RE, 2007. Improved pharmacokinetics of recombinant bispecific antibody molecules by fusion to human serum albumin. J. Biol. Chem 282, 12650–12660. 10.1074/jbc.M700820200. [PubMed: 17347147]

Nahar M, Dutta T, Murugesan S, Asthana A, Mishra D, Rajkumar V, Tare M, Saraf S, Jain NK, 2006. Functional polymeric nanoparticles: An efficient and promising tool for active delivery of bioactives. Crit. Rev. Ther. Drug Carrier Syst 23, 259–318. 10.1615/critrevtherdrugcarriersyst.v23.i4.10. [PubMed: 17341200]

Nathan DM, 2015. Diabetes: Advances in diagnosis and treatment. JAMA 314, 1052–1062. 10.1001/jama.2015.9536. [PubMed: 26348754]

Nauck MA, Wefers J, Meier JJ, 2021. Treatment of type 2 diabetes: Challenges, hopes, and anticipated successes. Lancet Diabetes Endocrinol. 9, 525–544. 10.1016/S2213-8587(21)00113-3. [PubMed: 34181914]

Nebert DW, Russell DW, 2002. Clinical importance of the cytochromes p450. Lancet 360, 1155–1162. 10.1016/S0140-6736(02)11203-7. [PubMed: 12387968]

Otsubo K, Yoneda T, Kaneko A, Yagi S, Furukawa K, Chuman Y, 2018. Development of a substrate identification method for human scp1 phosphatase using phosphorylation mimic phage display. Protein Pept. Lett 25, 76–83. 10.2174/0929866525666171206114913. [PubMed: 29210629]

Pasut G, Veronese FM, 2009. Peg conjugates in clinical development or use as anticancer agents: An overview. Adv. Drug. Del. Rev 61, 1177–1188. 10.1016/j.addr.2009.02.010.

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S, Habtemariam S, Shin HS, 2018. Nano based drug delivery systems: Recent developments and future prospects. J. Nanobiotechnology 16, 71–104. 10.1186/s12951-018-0392-8. [PubMed: 30231877]

Periti P, Mazzei T, Mini E, 2002. Clinical pharmacokinetics of depot leuprorelin. Clin. Pharmacokinet 41, 485–504. 10.2165/00003088-200241070-00003. [PubMed: 12083977]

Petersen MP, 2018. Economic costs of diabetes in the u.s. In 2017. Diabetes Care 41, 917–928. 10.2337/dci18-0007. [PubMed: 29567642]

Phaniendra A, Jestadi DB, Periyasamy L, 2015. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem 30, 11–26. 10.1007/s12291-014-0446-0. [PubMed: 25646037]

Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, Cunningham-Rundles C, Etzioni A, Holland SM, Klein C, Nonoyama S, Ochs HD, Oksenhendler E, Puck JM, Sullivan KE, Tang ML, Franco JL, Gaspar HB, 2015. Primary immunodeficiency diseases: An update on the classification from the international union of immunological societies expert committee for primary immunodeficiency 2015. J. Clin. Immunol 35, 696–726. 10.1007/s10875-015-0201-1. [PubMed: 26482257]

Poole RM, Nowlan ML, 2014. Albiglutide: First global approval. Drugs 74, 929–938. 10.1007/s40265-014-0228-2. [PubMed: 24861909]

Posma JJ, Grover SP, Hisada Y, Owens AP 3rd, Antoniak S, Spronk HM, Mackman N, 2019. Roles of coagulation proteases and pars (protease-activated receptors) in mouse models of inflammatory diseases. Arterioscler. Thromb. Vasc. Biol 39, 13–24. 10.1161/ATVBAHA.118.311655. [PubMed: 30580574]

Powell MF, Stewart T, Otvos L Jr., Urge L, Gaeta FC, Sette A, Arrhenius T, Thomson D, Soda K, Colon SM, 1993. Peptide stability in drug development. Ii. Effect of single amino acid substitution and glycosylation on peptide reactivity in human serum. Pharm. Res 10, 1268–1273. 10.1023/a:1018953309913. [PubMed: 8234161]

Puente XS, Sanchez LM, Overall CM, Lopez-Otin C, 2003. Human and mouse proteases: A comparative genomic approach. Nat. Rev. Genet 4, 544–558. 10.1038/nrg1111. [PubMed: 12838346]

Quatraro A, Consoli G, Magno M, Ceriello A, Giugliano D, 1989. Effectiveness of treatment of insulin-dependent diabetes with single dose of mixture of three kinds of insulin, employing semisynthetic human insulin. Diabete Metab. 15, 147–148. https://www.ncbi.nlm.nih.gov/pubmed/2668056. [PubMed: 2668056]

Rana S, Bajaj A, Mout R, Rotello VM, 2012. Monolayer coated gold nanoparticles for delivery applications. Adv. Drug. Del. Rev 64, 200–216. 10.1016/j.addr.2011.08.006.

Raptis S, Dimitriadis G, 1985. Human insulin. Clin. Physiol. Biochem 3, 29–42. https://www.ncbi.nlm.nih.gov/pubmed/3888492. [PubMed: 3888492]

Reddy RH, Kim H, Cha S, Lee B, Kim YJ, 2017. Structure-based virtual screening of protein tyrosine phosphatase inhibitors: Significance, challenges, and solutions. J. Microbiol. Biotechnol 27, 878–895. 10.4014/jmb.1701.01079. [PubMed: 28238001]

Rieckmann P, Zivadinov R, Boyko A, Selmaj K, Alexander JK, Kadosh S, Rubinchick S, Bernstein-Hanlon E, Stark Y, Ashtamker N, Davis MD, Khan O, 2021. Long-term efficacy and safety of three times weekly dosing regimen of glatiramer acetate in relapsing multiple sclerosis patients: Seven-year results of the glatiramer acetate low-frequency administration (gala) open-label extension study. Mult. Scler. J. Exp. Transl. Clin 7, 1–9. 10.1177/20552173211061550.

Roberts R, Smyth JW, Will J, Roberts P, Grek CL, Ghatnekar GS, Sheng Z, Gourdie RG, Lamouille S, Foster EJ, 2020. Development of plga nanoparticles for sustained release of a connexin43 mimetic peptide to target glioblastoma cells. Mater. Sci. Eng. C Mater. Biol. Appl 108, 110191. 10.1016/j.msec.2019.110191. [PubMed: 31923988]

Robinson NE, 2002. Protein deamidation. Proc. Natl. Acad. Sci. U. S. A 99, 5283–5288. 10.1073/pnas.082102799. [PubMed: 11959979]

Rohloff CM, Alessi TR, Yang B, Dahms J, Carr JP, Lautenbach SD, 2008. Duros technology delivers peptides and proteins at consistent rate continuously for 3 to 12 months. J. Diabetes Sci. Technol 2, 461–467. 10.1177/193229680800200316. [PubMed: 19885211]

Romancik JT, Gerber DG, Zhuang T, Cohen JB, 2022. Managing relapsed mantle cell lymphoma. Clin. Lymphoma Myeloma Leuk 10.1016/j.clml.2022.01.008.

Ronavari A, Igaz N, Adamecz DI, Szerencses B, Molnar C, Konya Z, Pfeiffer I, Kiricsi M, 2021. Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications. Molecules 26, 844–863. 10.3390/molecules26040844. [PubMed: 33562781]

Roopenian DC, Low BE, Christianson GJ, Proetzel G, Sproule TJ, Wiles MV, 2015. Albumin-deficient mouse models for studying metabolism of human albumin and pharmacokinetics of albumin-based drugs. MAbs 7, 344–351. 10.1080/19420862.2015.1008345. [PubMed: 25654695]

Rutman MP, Horn JR, Newman DK, Stefanacci RG, 2021. Overactive bladder prescribing considerations: The role of polypharmacy, anticholinergic burden, and cyp2d6 drugdrug interactions. Clin. Drug Investig 41, 293–302. 10.1007/s40261-021-01020-x.

Saeedi S, Israel S, Nagy C, Turecki G, 2019. The emerging role of exosomes in mental disorders. Transl Psychiatry 9, 122–133. 10.1038/s41398-019-0459-9. [PubMed: 30923321]

Sahin S, Benet LZ, 2008. The operational multiple dosing half-life: A key to defining drug accumulation in patients and to designing extended release dosage forms. Pharm. Res 25, 2869–2877. 10.1007/s11095-008-9787-9. [PubMed: 19015955]

Salazar AS, Rakhmankulova M, Simon LE, Toriola AT, 2021. Chemoprevention agents to reduce mammographic breast density in premenopausal women: A systematic review of clinical trials. JNCI Cancer Spectr. 5, 1–9. 10.1093/jncics/pkaa125.

Sani A, Cao C, Cui D, 2021. Toxicity of gold nanoparticles (aunps): A review. Biochem. Biophys. Rep 26, 100991–101003. 10.1016/j.bbrep.2021.100991. [PubMed: 33912692]

Sato AK, Viswanathan M, Kent RB, Wood CR, 2006. Therapeutic peptides: Technological advances driving peptides into development. Curr. Opin. Biotechnol 17, 638–642. 10.1016/j.copbio.2006.10.002. [PubMed: 17049837]

Schernthaner G, 1993. Immunogenicity and allergenic potential of animal and human insulins. Diabetes Care 16 Suppl 3, 155–165. 10.2337/diacare.16.3.155. [PubMed: 8299472]

Scotto R, Reia A, Buonomo AR, Moccia M, Viceconte G, Pisano E, Zappulo E, Brescia Morra V, Gentile I, 2021. Risk of invasive fungal infections among patients treated with disease modifying treatments for multiple sclerosis: A comprehensive review. Expert Opin. Drug Saf 925–936. 10.1080/14740338.2021.1918673. [PubMed: 33880975]

Serra Lopez-Matencio JM, Morell Baladron A, Castaneda S, 2018. Pharmacological interactions of monoclonal antibodies. Med. Clin. (Barc.) 151, 148–155. 10.1016/j.medcli.2017.10.037. [PubMed: 29269128]

Sheikh O, Yokota T, 2021. Developing dmd therapeutics: A review of the effectiveness of small molecules, stop-codon readthrough, dystrophin gene replacement, and exon-skipping therapies. Expert Opin. Investig. Drugs 30, 167–176. 10.1080/13543784.2021.1868434.

Sim RB, Tsiftsoglou SA, 2004. Proteases of the complement system. Biochem. Soc. Trans 32, 21–27. 10.1042/bst0320021. [PubMed: 14748705]

Singh R, Lillard JW Jr., 2009. Nanoparticle-based targeted drug delivery. Exp. Mol. Pathol 86, 215–223. 10.1016/j.yexmp.2008.12.004. [PubMed: 19186176]

Skwarecki AS, Nowak MG, Milewska MJ, 2021. Amino acid and peptide-based antiviral agents. ChemMedChem 16, 3106–3135. 10.1002/cmdc.202100397. [PubMed: 34254457]

Smith DA, Beaumont K, Maurer TS, Di L, 2018. Relevance of half-life in drug design. J. Med. Chem 61, 4273–4282. 10.1021/acs.jmedchem.7b00969. [PubMed: 29112446]

Snelder N, Drenth HJ, Riber Bergmann K, Wood ND, Hibberd M, Scott G, 2019. Population pharmacokinetic-pharmacodynamic modelling of the relationship between testosterone and prostate specific antigen in patients with prostate cancer during treatment with leuprorelin. Br. J. Clin. Pharmacol 85, 1247–1259. 10.1111/bcp.13891. [PubMed: 30731514]

Stamnaes J, Fleckenstein B, Sollid LM, 2008. The propensity for deamidation and transamidation of peptides by transglutaminase 2 is dependent on substrate affinity and reaction conditions. Biochim. Biophys. Acta 1784, 1804–1811. 10.1016/j.bbapap.2008.08.011. [PubMed: 18793760]

Swayzer DV, Gerriets V, 2021. Leuprolide, Statpearls, Treasure Island, Florida. https://www.ncbi.nlm.nih.gov/pubmed/31869126.

Swingle MR, Honkanen RE, 2019. Inhibitors of serine/threonine protein phosphatases: Biochemical and structural studies provide insight for further development. Curr. Med. Chem 26, 2634–2660. 10.2174/0929867325666180508095242. [PubMed: 29737249]

Tartibi HM, Hershfield MS, Bahna SL, 2016. A 24-year enzyme replacement therapy in an adenosine-deaminase-deficient patient. Pediatrics 137. 10.1542/peds.2015-2169.

Teixeira V, Feio MJ, Bastos M, 2012. Role of lipids in the interaction of antimicrobial peptides with membranes. Prog. Lipid Res 51, 149–177. 10.1016/j.plipres.2011.12.005. [PubMed: 22245454]

Todorova D, Simoncini S, Lacroix R, Sabatier F, Dignat-George F, 2017. Extracellular vesicles in angiogenesis. Circ. Res 120, 1658–1673. 10.1161/CIRCRESAHA.117.309681. [PubMed: 28495996]

Tomlinson S, Thurman JM, 2018. Tissue-targeted complement therapeutics. Mol. Immunol 102, 120–128. 10.1016/j.molimm.2018.06.005. [PubMed: 30220307]

Tornio A, Backman JT, 2018. Cytochrome p450 in pharmacogenetics: An update. Adv. Pharmacol 83, 3–32. 10.1016/bs.apha.2018.04.007. [PubMed: 29801580]

Toutain PL, Bousquet-Melou A, 2004. Plasma terminal half-life. J. Vet. Pharmacol. Ther 27, 427–439. 10.1111/j.1365-2885.2004.00600.x. [PubMed: 15601438]

Trujillo JM, Nuffer W, Smith BA, 2021. Glp-1 receptor agonists: An updated review of head-to-head clinical studies. Ther. Adv. Endocrinol. Metab 12, 1–15. 10.1177/2042018821997320.

Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P, 2002. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J. Biol. Chem 277, 14838–14843. 10.1074/jbc.M200581200. [PubMed: 11815627]

Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, Charlson F, Davis A, Degenhardt L, Dicker D, al., e., 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: A systematic analysis for the global burden of disease study 2013. Lancet 386, 743–800. 10.1016/S0140-6736(15)60692-4. [PubMed: 26063472]

Wallin MT, Culpepper WJ, Campbell JD, Nelson LM, Langer-Gould A, Marrie RA, Cutter GR, Kaye WE, Wagner L, Tremlett H, Buka SL, Dilokthornsakul P, Topol B, Chen LH, LaRocca NG, Workgroup, U.S.M.S.P., 2019. The prevalence of ms in the united states: A population-based estimate using health claims data. Neurology 92, e1029–e1040. 10.1212/WNL.0000000000007035. [PubMed: 30770430]

Werle M, Bernkop-Schnurch A, 2006. Strategies to improve plasma half life time of peptide and protein drugs. Amino Acids 30, 351–367. 10.1007/s00726-005-0289-3. [PubMed: 16622600]

Werner U, Haschke G, Herling AW, Kramer W, 2010. Pharmacological profile of lixisenatide: A new glp-1 receptor agonist for the treatment of type 2 diabetes. Regul. Pept 164, 58–64. 10.1016/j.regpep.2010.05.008. [PubMed: 20570597]

Wettergren A, Pridal L, Wojdemann M, Holst JJ, 1998. Amidated and non-amidated glucagon-like peptide-1 (glp-1): Non-pancreatic effects (cephalic phase acid secretion) and stability in plasma in humans. Regul. Pept 77, 83–87. 10.1016/s0167-0115(98)00044-5. [PubMed: 9809800]

Whayne TF, Saha SP, Mukherjee D, 2016. Antioxidants in the practice of medicine; what should the clinician know? Cardiovasc. Hematol. Disord. Drug Targets 16, 13–20. 10.2174/1871529×16666160614015533. [PubMed: 27296476]

Wintrich J, Kindermann I, Ukena C, Selejan S, Werner C, Maack C, Laufs U, Tschope C, Anker SD, Lam CSP, Voors AA, Bohm M, 2020. Therapeutic approaches in heart failure with preserved ejection fraction: Past, present, and future. Clin. Res. Cardiol 109, 1079–1098. 10.1007/s00392-020-01633-w. [PubMed: 32236720]

Wisniewski K, Qi S, Kraus J, Ly B, Srinivasan K, Tariga H, Croston G, La E, Wisniewska H, Ortiz C, Laporte R, Riviere PJ, Neyer G, Hargrove DM, Schteingart CD, 2019. Discovery of potent,

selective, and short-acting peptidic v2 receptor agonists. J. Med. Chem 62, 4991–5005. 10.1021/acs.jmedchem.9b00132. [PubMed: 31022340]

Wright HT, 1991. Sequence and structure determinants of the nonenzymatic deamidation of asparagine and glutamine residues in proteins. Protein Eng. 4, 283–294. 10.1093/protein/4.3.283. [PubMed: 1649998]

Wright JC, 2010. Critical variables associated with nonbiodegradable osmotically controlled implants. AAPS J. 12, 437–442. 10.1208/s12248-010-9199-8. [PubMed: 20490735]

Wu H, Huang J, 2018. Optimization of protein and peptide drugs based on the mechanisms of kidney clearance. Protein Pept. Lett 25, 514–521. 10.2174/0929866525666180530122835. [PubMed: 29848260]

Wynn DR, 2019. Enduring clinical value of copaxone(r) (glatiramer acetate) in multiple sclerosis after 20 years of use. Mult. Scler. Int 2019, 1–19. 10.1155/2019/7151685.

Xu W, Jimenez RB, Mowery R, Luo H, Cao M, Agarwal N, Ramos I, Wang X, Wang J, 2017. A quadrupole dalton-based multi-attribute method for product characterization, process development, and quality control of therapeutic proteins. MAbs 9, 1186–1196. 10.1080/19420862.2017.1364326. [PubMed: 28805536]

Yang H, Zubarev RA, 2010. Mass spectrometric analysis of asparagine deamidation and aspartate isomerization in polypeptides. Electrophoresis 31, 1764–1772. 10.1002/elps.201000027. [PubMed: 20446295]

Yang NJ, Hinner MJ, 2015. Getting across the cell membrane: An overview for small molecules, peptides, and proteins. Methods Mol. Biol 1266, 29–53. 10.1007/978-1-4939-2272-7_3. [PubMed: 25560066]

Yeo PL, Rabenstein DL, 1993. Characterization of the thiol/disulfide chemistry of neurohypophyseal peptide hormones by high-performance liquid chromatography. Anal. Chem 65, 3061–3066. 10.1021/ac00069a019. [PubMed: 8256869]

Young GA, Kendall S, Brownjohn AM, 1994. D-amino acids in chronic renal failure and the effects of dialysis and urinary losses. Amino Acids 6, 283–293. 10.1007/BF00813748. [PubMed: 24189736]

Yuan D, Zhao Y, Banks WA, Bullock KM, Haney M, Batrakova E, Kabanov AV, 2017. Macrophage exosomes as natural nanocarriers for protein delivery to inflamed brain. Biomaterials 142, 1–12. 10.1016/j.biomaterials.2017.07.011. [PubMed: 28715655]

Zaman R, Islam RA, Ibnat N, Othman I, Zaini A, Lee CY, Chowdhury EH, 2019. Current strategies in extending half-lives of therapeutic proteins. J. Control. Release 301, 176–189. 10.1016/j.jconrel.2019.02.016. [PubMed: 30849445]

Zhang Y, Sun Z, Jia J, Du T, Zhang N, Tang Y, Fang Y, Fang D, 2021. Overview of histone modification. Adv. Exp. Med. Biol 1283, 1–16. 10.1007/978-981-15-8104-5_1. [PubMed: 33155134]

Zhao Y, Yang Y, Loscalzo J, 2014. Real-time assessment of the metabolic profile of living cells with genetically encoded nadh sensors. Methods Enzymol. 542, 349–367. 10.1016/B978-0-12-416618-9.00018-2. [PubMed: 24862275]

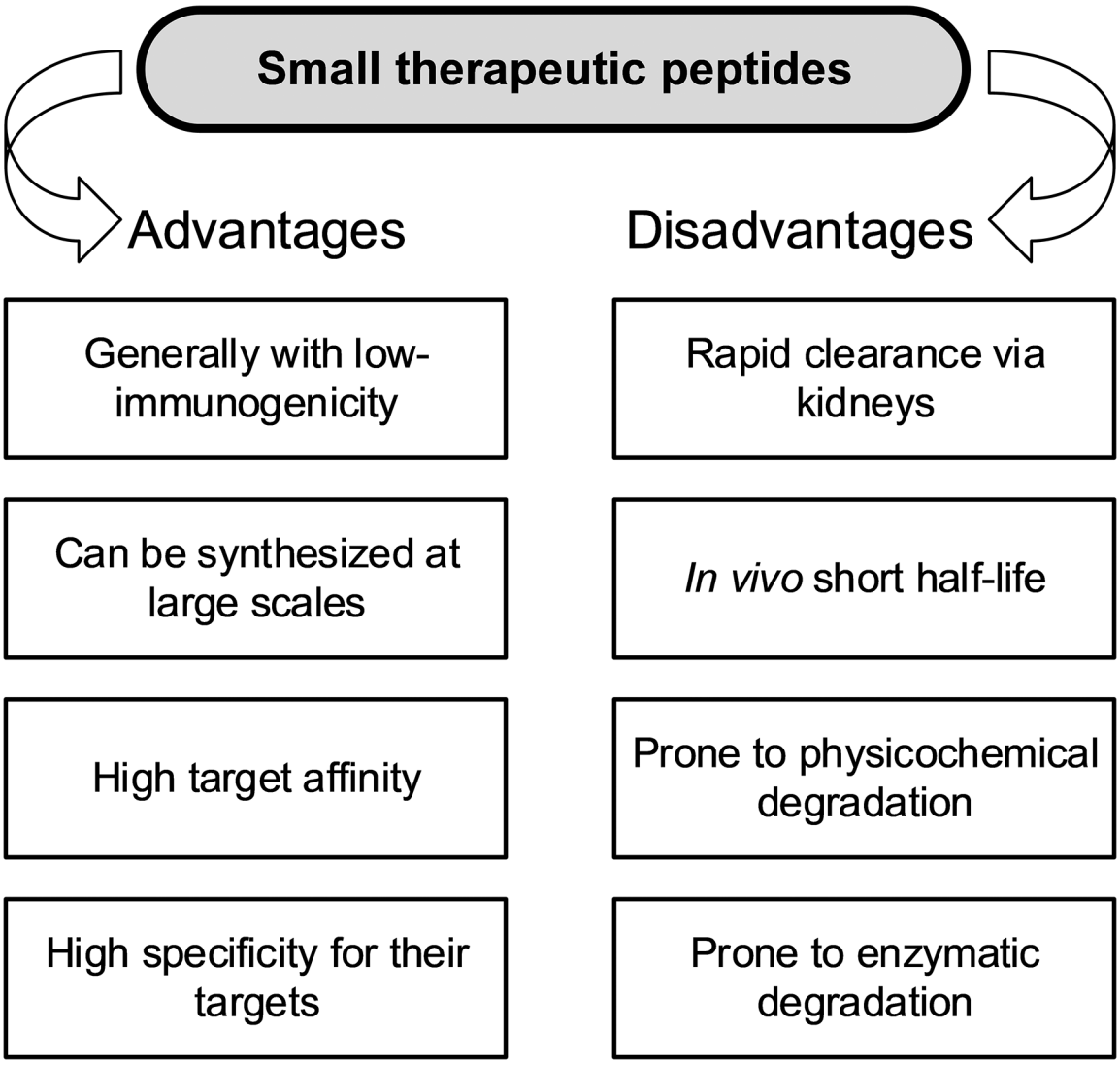

Fig. 1.
Advantages and disadvantages of small therapeutic peptides.

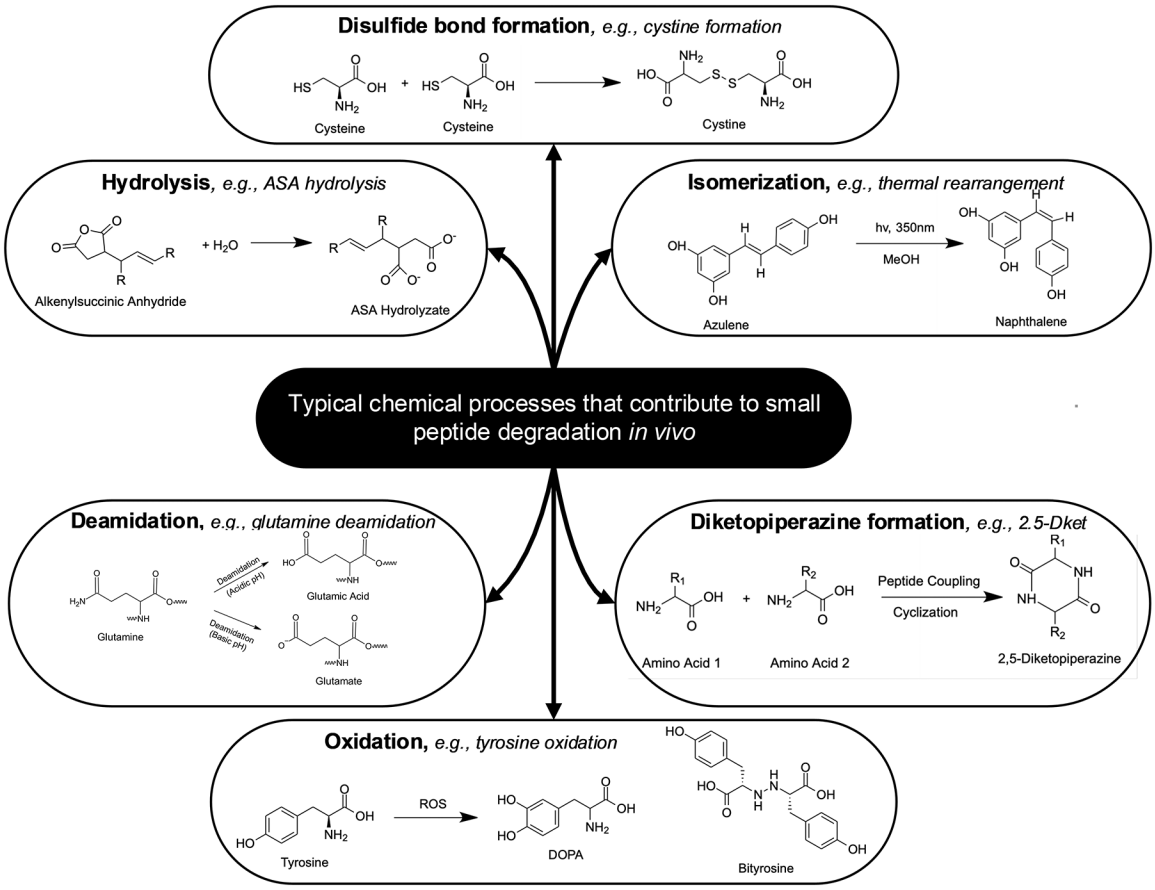

Fig. 2.

Major forms of chemically-associated *in vivo* degradations of small peptides. The formulas were drawn using the ChemSketch freeware (Version 8.0) developed by Advanced Chemistry Development (Toronto, ON, Canada; https://www.acdlabs.com)

Table 1

Current methods and emerging tactics to protect small peptides and elongate their half-lives.

Method	Major technical point	Example	Advantage	Disadvantage	Reference
Current Method	N- or C-terminus modification	N-acetylated GLP-1 (in development)	Protection against exogenous proteases	Modifications may introduce a decrease in binding affinity	(John et al., 2008; Kim et al., 2013)
	Replacing L-AA(s) with D-AA(s)	Desmopressin	Longer half-life	Modification may introduce a decrease in binding affinity	(Agerso et al., 2004; Wisniewski et al., 2019)
	Conjugated with macromolecule (*e.g.*, PEG)	PEG conjugated adenosine deaminase (enzyme)	Protection from degradation *in vivo*, longer half-life.	PEG may cause hypersensitivity, unexpected changes in the pharmacokinetics of the peptide and unwanted accumulation in the body due to being non-degradable.	(Knop et al., 2010; Tartibi et al., 2016)
	Conjugated with macromolecule (*e.g.*, albumin)	Albiglutide (Tanzeum®)	Protection from degradation *in vivo*, longer half-life.	Conjugation with a macromolecule may cause unexpected changes in the pharmacokinetics of the peptide.	(Bronden et al., 2017; Poole and Nowlan, 2014)
	Drug delivery vehicle	Leuprolide (leuprorelin or Lupron®) with drug delivery vehicle DUROS®	Allows administration of the peptide over a long period; protects the peptide from physical and chemical degradation; decreases the frequency of administration.	The peptide should be dissolved in a solvent that can pass through the semi-permeable membrane.	(Crawford et al., 2015; Fowler et al., 2000; Rohloff et al., 2008)
Emerging Tactic	Exosome	There is no FDA-approved example	Non-toxic; already found *in vivo*; have high target specificity and can penetrate through cell membrane.	Encapsulating peptides into exosomes is challenging	(Bunggulawa et al., 2018; Marsh et al., 2021b)
	Conjugated with nanoparticle	Gold nanoparticles	Easy to synthesize; can pass through cellular membranes easier than macromolecules.	Toxicity of these nanoparticles are not fully understood; even though gold nanoparticles are FDA approved, there is evidence of potential toxicity.	(Ronavari et al., 2021; Sani et al., 2021)
	Protease and phosphatase inhibitors	Saquinavir (Invirase®), ritonavir (Norvir®), elbasvir/grazoprevir (Zepatier®)	Some protease inhibitors are being used to treat HIV and hepatitis C, so they are highly optimized for human use and side effects are known.	Inhibiting proteases and phosphatases *in vivo* can cause unwanted side effects and toxicity, as these enzymes are required in various systems *in vivo* such as signaling cascades and immune response.	(De Leuw and Stephan, 2018; Festa et al., 2021)

Abbreviations: AA(s): amino acid(s); FDA: food and drug administration (US); GLP-1: glucagon-like peptide 1; PEG: polyethylene glycol; HIV: human immunodeficiency virus

Table 2

Examples of small therapeutic peptides that are in clinical use or are currently under testing in clinical trials.

Brand name [Company name]	Generic name	Size (AAs)	Disease	Delivery method	Anti-degradation strategy	Status	Reference
Various, *e.g.*, Humulin R® [Novo Nordisk, Sanofi, Eli Lilly]	Various, *e.g.*, insulin regular	51	Type 1 diabetes	i.v.	Acylation, substitutions or additions of AAs	Routinely used in clinical therapy	(Kjeldsen et al., 2021; Kurtzhals et al., 2021)
Adlyxin® (*i.e.*, Lyxumia®) [Sanofi]	Lixisenatide	44	Type 2 diabetes	i.v.	C-terminus amidation	Routinely used in clinical therapy	(Baker and Levien, 2017; Meier, 2012; Trujillo et al., 2021; Werner et al., 2010)
Bydureon® (*i.e.* Byetta®) [AstraZeneca]	Exenatide	39	Type 2 diabetes	i.v.	Encapsulation in microspheres	Routinely used in clinical therapy	(Genovese et al., 2017; Mathieu et al., 2013)
Plenaxis® [Praecis Pharmaceuticals]	Abarelix	10	Prostate cancer	i.v.	5 non-natural AAs used in its structure.	Used in clinical therapy in Germany & Netherlands	(Garnick and Mottet, 2012; Mongiat-Artus and Teillac, 2004; Moul, 2014)
Velcade® [Millennium Pharmaceuticals]	Bortezomib	2	Multiple myeloma & mantle cell lymphoma	i.v.	N-terminus modification	Routinely used in clinical therapy	(Mahmoudian et al., 2021; Romancik et al., 2022)
Copaxone® [Teva Pharmaceutical Industries]	Glatiramer acetate	10	MS	i.v.	administration along with mannitol as a vehicle	Routinely used in clinical therapy	(McGinley et al., 2021; Scotto et al., 2021)
Lupron® [AbbVie]	Leuprolide (or leuprorelin)	9	Breast & prostate cancer	i.v.	Encapsulated in biodegradable lipophilic synthetic microspheres and nonbiodegradable implant	Used in clinical therapy	(Salazar et al., 2021; Swayzer and Gerriets, 2021)
Lupron® [AbbVie]	Leuprolide (leuprorelin)	9	Central precocious puberty	i.v.	Encapsulated in biodegradable lipophilic synthetic microspheres and nonbiodegradable implant	Phase III clinical trials	(Bereket, 2017; Mejia-Otero et al., 2021; Periti et al., 2002)
Natrecor® [Scios]	Nesiritide	32	Decompensated heart failure	i.v	Administration in hospital setting	Used in clinical therapy	(Elkayam et al., 2002)
Granexin® [FirstString Research]	αCT1	25	Diabetic foot ulcers, cutaneous scar reduction & skin radiation injury	Topical	Topical application in gel form	Phase II & III clinical trials	(Ghatnekar et al., 2015; Ghatnekar et al., 2009; Montgomery et al., 2018)

Abbreviations: αCT1: alpha-carboxy terminus 1; AA(s): amino acid(s); i.v.: intravenous injection; MS: multiple sclerosis