PEPTIDES: A NEW THERAPEUTIC APPROACH

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ABSTRACT

Peptide therapeutics have played a notable role in medical practice since the advent of insulin therapy in the 1920s. Peptide drugs are approved in the United States and other major markets, and peptides continue to enter clinical development at a steady pace. Peptide drug discovery has diversified beyond its traditional focus on endogenous human peptides to include a broader range of structures identified from other natural sources or through medicinal chemistry efforts. Peptides are recognized for being highly selective and efficacious and, at the same time, relatively safe and well tolerated. Consequently, there is an increased interest in peptides in pharmaceutical research and development (R and D), and approximately 140 peptide therapeutics are currently being evaluated in clinical trials. Given that the low-hanging fruits in the form of obvious peptide targets have already been picked, it has now become necessary to explore new routes beyond traditional peptide design. Examples of such approaches are multifunctional and cell-penetrating peptides, as well as peptide drug conjugates. In regards to patient compliance for drug delivery, oral drug delivery is generally the preferred route of administration. However, parental injection of peptide drugs has always been the primary method of peptide drug administration. Nevertheless, oral delivery of peptide drug presents a significant challenge due to the enzymatic degradation by enzymes in the GI tract and the poor penetration of the peptides across gastrointestinal epithelium membranes, particularly for adults. Therefore, a novel peptide drug analogue or pro-drug that both protect peptide drugs from degradation by the enzymes in the GI tract and also improves its penetration across the intestinal epithelium membrane would greatly advance the development of peptide drugs as effective candidates for the treatment of various diseases.

Keywords: Peptides, Medicinal chemistry, Drug Conjugates, Cell-penetrating Peptides

INTRODUCTION

Peptides are composed of short chains of amino acid monomers linked together via peptide bonds and occur naturally in the human body. Peptides are very specific in activity when compared to small molecules when used as a drug candidate. Generally having fewer side effects, peptides have become popular candidates for drug design. In 2007, there are about 60 approved peptide drugs that are in clinical use and have generated approximately $13 billion USD as of 2010. Another 140 peptide candidates are in clinical trials, as well as another 500 to 600 in pre-clinical development. The difficulty associated with marketing peptide drugs, however, is the low oral bioavailability as a result of physical and biochemical barriers of the gastrointestinal tract forcing invasive parental delivery methods to be the only practical method of delivery. Such parental injection methods include intravenous, intramuscular and subcutaneous injections. Unfortunately, parental delivery methods make administration of peptide drugs difficult and painful, which leads to lower patient compliance and ultimately, the reduced popularity of using peptide drugs on a frequent basis. Oral administration, on the other hand, would offer easy, a convenient administration that can be sold over the counter [1].

Oral administration of peptide drugs is severely hindered by the physical, biological and chemical barriers of the gastrointestinal (GI) tract. Such chemical, biological and physical barriers present in the GI tract serve to primarily protect the body from pathogens, antigens or any other harmful substances while allowing both digestion and absorption of ingested nutrients or fluids for essential body functions. The chemical barrier for peptide drug delivery is attributed to the proteases and the low pH environments of the stomach that are both essential for the digestion of proteins required for the successful absorption of amino acids [2].

The utilization of peptides as therapeutics has evolved over time and continues to evolve with changes in drug development and treatment paradigms. Peptides isolated from natural sources, such as insulin and ACTH, provided life-saving medicines in the first half of the 20th century. When sequence elucidation and chemical synthesis of peptides became feasible in the 1950s, synthetic oxytocin and vasopressin also entered clinical use. As venoms of arthropods and cephalopods became recognized as treasure troves of bioactive peptides, isolation of natural products from exotic sources became a popular strategy for identifying new potential therapeutics. The genomic era allowed for the identification and molecular characterization of receptors for many important endogenous peptide hormones, and industry and academia began to pursue novel peptidic ligands for these receptors [3].

These therapeutics tend to have high molecular weights, low lipophilicity and charged functional groups that hamper their absorption. These characteristics lead to the low bioavailability of most orally administered peptides (<2%) and short half-lives (<30 min). Intravenous (iv.) or subcutaneous (sc) delivery of these therapeutics overcomes the issue of absorption, but other factors limit the bio-availability of peptide and protein therapeutics including systemic proteases; rapid metabolism; opsonization; conformational changes; dissociation of subunit proteins; non-covalent complexation with blood products; and destruction of labile side-groups. As oral delivery improves patient compliance, there is great interest in the development of systems that allow for the oral delivery of peptide and protein therapeutics [4].

Furthermore, the availability of massive combinatorial chemistry libraries and high-throughput screening (HTS) technologies swung the pendulum in a new direction, towards small molecules that target peptide receptors. Small molecules are generally more suitable for oral delivery and easier to manufacture than peptides; the challenge lies in finding a small molecule that mimics a peptide ligand’s receptor binding and selective modulation. The number and diversity of scaffolds present in modern screening libraries supported the idea that lead molecules could be identified, optimized, and developed into drugs. Structural biology added another arrow to the quiver by elucidating key molecular interactions at receptor active sites that could be leveraged by any class of molecule. The small molecule approach has been more successful in some cases than others. At peptidic receptors, small
molecules are often less potent than peptides, and small molecules that act as antagonists are easier to identify than agonists. The large ligand-binding site of some peptide GPCRs and specific conformational change required for signal transduction provide significant challenges for small molecule drug discovery, particularly for Class B GPCRs [5].

Nonetheless, orally available small molecules such as losartan and valsartan replaced the peptide saralasin (SARENIN) as angiotensin II receptor blockers for hypertension, and other small molecule drugs target Class A GPCRs for which no peptide drugs are marketed. While overcoming some of the challenges of peptide drugs, these small molecules retain the potential for liabilities that are infrequently associated with peptides, such as CYP inhibition leading to drug-drug interactions (DDIs) and side effects caused by off-target binding. Although a significant improvement, a major challenge of new discovery, small molecule ligands for peptide receptors are not a complete substitute for peptide compounds [6].

**Different routes of delivery**

**Oral delivery**

Oral delivery is the preferred route of drug administration, as the majority of patients see it as the most convenient way to take their drugs. Drugs taken by the oral route have the highest level of patient compliance due to the ease and simplicity of taking medications.

Despite a large number of protein therapeutics being discovered each year, oral delivery continues to be a barrier. As a whole, protein and peptide drugs have low bioavailability when administered orally due to problematic barriers including gastrointestinal proteases, the epithelial barrier and efflux pumps [7].

**Strategies for oral delivery of peptides**

**Chemical modifications**

Peptide analogues or peptidomimetics are peptide sequences utilizing unnatural amino acids or unnatural peptide bond linkages between amino acids. Such modifications create a resulting peptide sequence that is less susceptible to enzymatic degradation. As naturally occurring proteases are designed to catalyse reactions involving natural peptides and natural peptide bonds, one difficulty in this approach is the activity of the drug must be retained.

Unnatural amino acids and unnatural steric isomers of a peptidomimetic are required to be able to interact with the original intended receptor or targets. N-alkylation and a-alkylation of amino acids can provide steric hindrance against enzymatic degradation. Modification of peptide bonds can create bonds between amino acids that are resistant to peptidases that cleave peptides at peptide bonds to liberate amino acids. Examples of biologically active and enzymatically stable peptide bond substitutes previously used include: reduced amide bond, alkene, hydroxy alkene, hydroxy ethylamino, dihydroxy ethylene and thioamides [8]. Reversal of stereochemistry from natural D-amino acids to L-amino acids has shown to increase resistance to proteases while retaining activity. Increase in lipophilicity or decrease in hydrogen bonding potential by chemical modification of a peptide can improve the cell penetrating ability of a peptide. It has been shown that a chain of methyl phenylalanine had improved caco-2 cell culture penetration compared to the same peptide chain of phenylalanine [9].

**Peptide pro-drug conjugates**

Pro-drugs are conjugates of drugs that can be easily metabolized using enzymes in the human body or under physiological conditions to release the natural drug and non-toxic by-products. Pro-drugs for oral peptide delivery are designed to remain in the inactive pro-drug form while in the GI tract to be protected from degradation in GI conditions. Pro-drugs can also improve the physical properties of a drug to increase uptake through the intestinal cell membrane. After bypassing these barriers, the drug is released from the pro-drug by metabolism. Therefore, readily cleavable linkers have been developed to maximise the drug recovery rate within the body.

Lipophilic moieties such as long fatty acid chains have been common conjugates used for increasing the lipophilicity of hydrophilic peptides to enhance uptake. The conjugation of palmitic acid to Leucine5-enkephalin via an ester bond combined with the use of nanoparticle GCQ formulation methods has shown an increase in activity and duration of effect compared to the unconjugated peptide in the same nanoparticle formulation [10].

**Pegylation**

Pegylation has also been used as a systemic stability enhancer. Direct Pegylation can aid in the stability of proteins for delivery, mainly leading to an increase in circulation time. PEG molecules are highly hydrated, and this increased size leads to decreased glomerular filtration. Moreover, pegylation of proteins is thought to reduce protein aggregation and opsonization. Pegylation also reduces uptake by the RES, decreases formation of antibodies against the protein and decreases the apparent volume of distribution. Pegylation, however, does have drawbacks. Due to the size of PEG, steric hindrance may decrease the activity of the protein. Also, increased protein aggregation after pegylation has been noted. Chronic iv. Administration of PEG proteins has unintended consequences such as vacuolation of the renal cortical tubular epithelium in laboratory animals. However, these side effects were noted only after exposure to toxic, supratherapeutic doses of PEG. Newer pegylation methods such as living radical polymerization, free radical polymerization, atom transfer radical polymerization and reversible addition fragmentation transfer have allowed pegylation with greater specificity and purity while making a modification with PEG a simpler task [11].

**Absorption enhancers**

Another method used to improve peptide oral bioavailability is the co-administration of absorption enhancers. Absorption enhancers are a wide range of chemical compounds through a wide range of mechanisms. Absorption enhancers that have been reported in the literature with some success include: ethylenediaminetetraacetic acid (EDTA), citric acid, salicylate, N-acyl derivatives of collagen, cyclodextrins, sodium caprylate, sodium lauryl sulphate and sodium taurocholate. Absorption enhancements act via different mechanisms to increase the penetration of peptides through intestinal cell membranes. Mechanisms of action for absorption enhancers include: opening tight junctions, changing the membrane fluidity and changing the mucous viscosity [12].

The optimal absorption enhancer should be reversible, nontoxic at the effective concentration and provide a rapid permeation enhancing the effect on the intestinal cell membrane. One such compound class of absorption enhancers is chitosans. Chitosans are nontoxic, biocompatible, FDA-approved polymer derivatives of chitin that enhance the absorption of hydrophilic macromolecule drugs. In addition, due to their high molecular weight, they are minimally absorbed from the gut, limiting the possibility of systemic side effects. It is thought that varying degrees of deacetylation of chitin confer different amounts of absorption enhancement, with 80% deacetylation affording the greatest promoter effect in cell culture. Chitosans have been used to enhance the absorption of molecules such as atenolol, insulin and B-R-vasopressin. Further, chitosans appear to be quite safe at their effective concentration [13].

**Enzyme inhibitors**

Enzymatic inhibitors, as the name suggests, are capable of inactivating certain enzymes. Co-administration of enzyme inhibitors specific to the inactivation of GI peptidases that catalyse the metabolism of the administered peptide drug with the administration of peptide drugs can serve to decrease degradation of peptides in the GI tract and hence increase the oral bioavailability of peptide drugs. Enzyme inhibitors for peptide drug delivery can be classified into polypeptide protease inhibitors, peptides, amino acids, and inhibitors that are not based on amino acids [14].

**Microemulsions**

Microemulsions are defined as isotropic, thermodynamically stable transparent systems consisting of oil, water, surfactant and sometimes, co-surfactant forming particles with a droplet size of <200 nm. Microemulsions are typically classified into three classes or a combination of the three classes: oil-in-water (o/w), water-in-
oil (w/o) and bicontinuous. The ratio of oil phase, aqueous phase, surfactant and in some cases the co-solvent in an emulsion determines the resulting type of emulsion formed. The type of microemulsion formed is also dependant on the type of surfactant used. Surfactants with a hydrophilic-lipophilic balance (HLB) value>12 primarily favours the formation of o/w emulsions whereas surfactants with a HLB value<12 favours the formation of w/o emulsion. The main advantages of microemulsions over colloidal systems such as suspensions and emulsions include low viscosity, higher stability, improved solubility, ease of manufacturing, ease of upscale and improved bioavailability [15].

Nanoparticles
Nanoparticles (NPs) are solid particles with sizes in the range of 10–1000 nm. NPs allow for the encapsulation of proteins inside a polymeric matrix, thus protecting them against hydrolysis and enzymatic degradation. These systems can be tuned in order to maximize encapsulation efficiency, bioavailability and retention time. NPs, however, have a difficult time being absorbed from the GI tract; studies have demonstrated that cells lacking mucus (including M cells and Peyer’s patches in general) are best at absorbing NPs. Particles of 50 and 100 nm demonstrated the greatest absorption and detection in the intestinal mucosa. Furthermore, NPs smaller than 100 nm demonstrate a higher extent of uptake by absorptive enterocytes while those over 500 nm will rarely be taken up by absorptive enterocytes [16]. NPs are often made from poly(lactic acid), poly(lactic-co-glyclic acid), chitosan, gelatin and poly-alkylacyanoacrylate, all of which are non-toxic, non-thrombogenic, non-immunogenic, non-inflammatory, stable in blood, biodegradable, avoid the reticuloendothelial system (RES), and are applicable to various biologics such as proteins, peptides and nucleotides. While there are minimal scientific data on the toxicity of NPs, their size makes exposure during manufacturing almost guaranteed [17].

Liposomes
While liposome systems have potential in oral drug delivery, there is a concern with the stability of the vesicles under the physiologic conditions of the GI tract. Adding to the problem, mucus may act as a barrier by blocking the diffusion of liposomes to the epithelial layer. Despite this, orally administered liposomes have demonstrated some successes. Calcitonin was administered in a chitosan–aprotinin coated liposome and illustrated an increased pharmacological effect compared with free calcitonin. Cyclosporine has also been delivered in liposomes; the egg lectin–cremophore–lactose liposome containing cyclosporin had nine-times the bioavailability of free cyclosporin and four-times that of the micro emulsion on the market. PEG coating, enteric encapsulation and the use of archaeosomes have been proposed to decrease degradation of the liposome in the GI tract [18].

Mucosal delivery systems
Most mucosal delivery systems are formulated by using mucosal micelles. Mucosal micelles are multi-functional macromolecules, which in addition to their mucosal mucoadhesive properties increase the permeability of the drug candidates across epithelial membranes and simultaneously inhibit peptidolytic enzymes. These polymers make close contact with the mucosal layer and therefore exert their effects within a limited area of the intestinal mucosa. Some of the mucoadhesive polymer/copolymers that have shown excellent bioadhesive properties include sodium carboxymethylcellulose, polyacryclic acid, tragacanth, polymethyl vinyl ether-co-maleic anhydride, polyethylene oxide and methylcellulose. Mucosal delivery systems have increased the residence time of the system at the absorptive mucosal membrane, leading to increased time available for absorption to occur and hence improved absorption of proteins and peptides [19].

Therapeutic uses of peptides
Peptides have been investigated across the therapeutic spectrum, reflecting the potential utility across a wide range of indications and perhaps coupled with the cautious optimism that accompanies many development programs. Not surprisingly, the areas of highest concentration for peptide development (at present) are areas of high interest to the pharmaceutical industry: metabolic disease, oncology, and cardiovascular disease. Interestingly, the therapeutic landscape of approved peptide drugs does not mirror that of peptides in development. For example, many peptides have entered development in oncology indications but few have received approval, which may simply reflect poor success rates in oncology as a whole [20].

Emerging peptide areas and technologies
There is a large pool of natural peptides, some of which represent excellent starting points for therapeutics. In the metabolic area, for example, the gut, the micro biome has received much interest because it is rich in diverse bacteria that could give rise to the identification of new peptides from protein fragments, degradation products, or signalling molecules. Among the emerging technologies
the field are multifunctional peptides representing more than one pharmacological activity, such as dual or even triple agonism. This approach makes sense based on information from genomics. Thus, it is evident that knockout animals, where only a single gene is deleted, often present with no distinct phenotype. Also, despite broad industrial efforts of the G-protein coupled receptors (GPCR) field that have led to the successful identification of several selective agonists and antagonists in clinical development, only a few ligands resulted in approved medicines. These lessons point to the redundancy in biological systems and favor multitarget approaches for the development of medicines. Another aspect of applying a polypharmacological approach is the possibility of more individualized and personalized treatment of differentiated patient groups [21].

Current multifunctional peptides in development include antimicrobial peptide drug candidates that have additional biological functions, such as immune stimulation and wound healing. Also, the trend towards multifunctional peptides can be seen in the GLP-1 agonist field, which represents an established drug class with several products, including exenatide, lixisenatide, and most recently albiglutide, being introduced to the market with great commercial success. Looking across clinical and preclinical pipelines, it is evident that several companies have focused on the development of GLP-1 dual and even triple agonists for a more diversified and personalized treatment of type-2 diabetes mellitus (T2DM) and/or obesity. It is also evident that, in addition to multifunctional peptides, there is a focus on improved patient convenience and compliance and, therefore, strategies towards less-frequent dosing, or even oral administration of GLP-based drugs, being pursued in clinical development [22].

Examples of other peptide compounds or modules that have been combined with GLP-1 agonism include glucagon (GCG), glucose-dependent insulinotropic peptide (GIP), cholecystokinin B (CCKB), and glucagon-like peptide 2 (GLP-2). The most clinically advanced multifunctional peptides are GLP-1–GIP and GLP-1–GCG dual agonists, which are currently being investigated in clinical proof-of-concept studies in patients who are overweight and have diabetes. The GLP-1–GCG dual agonists (some of which are modulated over the natural dual-acting gut peptide oxyntomodulin) are expected to provide a greater weight loss in overweight patients with T2DM compared with a pure GLP-1 agonist, via a GCG-derived increase in energy expenditure [23].

Another example is the GLP-1–CCKB dual agonists where the addition of CCKB (gastrin) agonism to the GLP-1 action is aimed at enhancing the expression of the pancreatic beta cell function, which in turn might aid in minimizing or preventing disease progression in T2DM. These examples illustrate how the addition of a second activity to the established effects of GLP-1 could lead to more individualized medical solutions with increased efficacy [24].

The development of multifunctional peptides could present a challenge in the sense that the prediction of the in vivo outcome for the drug candidates is more complex with dual target pharmacology versus single target pharmacology. One challenging aspect of the translation from in vitro to in vivo effects is the potentially biased signalling that might arise from novel ligands aimed at two or more receptors. In addition, the translation of results from animal models to human situations might be associated with greater risk for multifunctional peptides compared with single receptor peptides, because the uncertainty from two or more targets is multiplied. In the antibody field, similar challenges have been observed in the development of bispecific antibodies for the treatment of cancer. For these reasons, it is relevant to expect that multifunctional peptides might arise mainly from established paradigms, as observed in the GLP-1 field, more than from completely novel peptide combinations [25].

Clinical development timelines and benchmark for peptides

The duration of peptide clinical development has varied widely for the peptides approved since the beginning of 2010. The median development time for this cohort of peptides was 9.4 yr, which is slightly longer than one benchmark for cycle times (median of 8.1 yr) that captures data from a group of primarily mid-to-large-sized pharmaceutical companies across all molecules. In general, peptides with a shorter length of clinical development were approved in indications for which clear regulatory precedent and well-defined clinical trial endpoints exist: secondary hyperparathyroidism (etelcalcetide), type 2 diabetes mellitus (dulaglutide and albiglutide), and multiple myeloma (carfilzomib). These peptides were also typically advanced through mid-to-late-stage clinical trials by larger drug sponsors. The peptide rates of success fell between those of new biological entities (NBEs) and new chemical entities (NCEs) as described by CMR International. This may reflect the increased target specificity and reduced toxicity of peptides compared to small molecules, which have high attrition rates in early clinical development. In contrast, peptides may be less stable and less specific than protein biologics, including highly-target-selective monoclonal antibodies and prophylactic vaccines, resulting in increased attrition rates compared to NBEs [26].

Abbreviated new drug applications (ANDA) for certain highly purified synthetic peptide drug products

Submission of an ANDA for a proposed generic synthetic peptide for which the reference listed drug (RLD) is a peptide of rDNA origin generally would be appropriate if, among other things, the applicant can: 1) show that, for each peptide-related impurity that is found in both the proposed generic synthetic peptide and the RLD, the level of impurity in the proposed generic synthetic peptide is the same as or lower than that found in the RLD; 2) show that the proposed generic synthetic peptide does not contain any new specified peptide-related impurity that is more than 0.5 percent of the drug substance; 3) characterize each new specified peptide-related impurity; and 4) justify for each new specified peptide-related impurity that is no more than 0.5 percent of the drug substance why such impurity does not affect the safety of the proposed generic synthetic peptide and does not affect its effectiveness.

ANDA applicants are encouraged to apply orthogonal analytical methods to characterize the following properties and other properties, as appropriate:

- Primary sequence and physicochemical properties
- Secondary structure
- Oligomer/Aggregation states
- Biological activities (by in vitro or animal studies)

Where data demonstrate that the proposed synthetic peptide’s active ingredient is the “same as” the active ingredient in the reference listed drug (RLD), whether an application should be submitted as an ANDA or as an application submitted pursuant to section 505(b)(2) of the Food, drug and cosmetic act (FD and C Act), may depend on the product’s therapeutic profile, because differences in impurities may affect, among other things, the potential for immunogenicity.

In reviewing an ANDA, Food, Drug and Administration (FDA) consider the types and amounts of impurities present in a proposed generic drug in comparison to its RLD. In general, a proposed generic synthetic peptide should not contain impurities at levels greater than those found in the RLD. Any impurities, including new impurities, should be justified to help ensure, among other things, that the generic drug does not pose a greater safety risk, including with respect to immunogenicity, than the RLD.

Based on an understanding of the peptide-related impurities that could be present in the peptides, and given current analytical capabilities and current manufacturing capabilities to control peptide-related impurities, FDA believes that filing of an ANDA for the peptides covered would be generally appropriate if, among other things, the new specified peptide-related impurity level for the proposed generic synthetic peptide is no more than 0.5 percent of the drug substance. A new specified peptide-related impurity level higher than 0.5 percent of the drug substance raises concerns about the potential risk of immunogenicity that FDA believes could not be adequately addressed in an ANDA (e.g., assessment of the risk of immunogenicity would require clinical data under these circumstances).
A new specified peptide-related impurity level of no more than 0.5 percent of the drug substance for purposes of filing an ANDA is consistent with the small amount of unspecified peptide-related impurities observed in finished peptide drug products due to batch-to-batch variability, which occurs regardless of whether the peptide is produced by a recombinant or synthetic process. This allowance is, however, subject to subsequent scientific review upon the filing of an ANDA and FDA may ask the ANDA applicant to further reduce the level of a specified peptide-related impurity depending on the risks associated with a particular impurity as well as with the proposed drug product.

For each new specified peptide-related impurity that is not more than 0.5 percent of the drug substance, the ANDA applicant should characterize the impurity. Further, the ANDA applicant should provide justification for why such impurity does not affect the safety of the proposed generic synthetic peptide (including with respect to immunogenicity) and why it does not affect its effectiveness. This justification should take into consideration, among other things, the identity and amount of an impurity, the impurity’s impact on the physicochemical and biological properties of the peptide, and the potential risks specific to the peptide [27].

**THPdb: database of FDA-approved peptide and protein therapeutics**

Over the last decade, a plethora of databases encompassing information on protein and peptides with different therapeutic functionalities have been developed, reflecting the increased interest for proteins and peptides as therapeutics among the scientific community. A substantial interest has also been shown towards peptide-based subunit vaccine and immunotherapeutic. The information on US-FDA approved protein and peptide therapeutics along with their pharmacokinetic/pharmacodynamics properties, their advantages, chemical modifications, and their limitations are very important. However, this information is not easily accessible and it is scattered in the literature. To the best of authors’ knowledge, to date, no single freely available platform exists which is totally dedicated to US-FDA approved protein and peptide therapeutics. Therefore, keeping the above facts in mind, THPdb was developed, which is a comprehensive resource of all US-FDA approved protein and peptide therapeutics along with their corresponding drug variants available in the market. It is anticipated that the information available in the THPdb is very helpful to the researchers working in the field of peptide and protein-based drug discovery [28].

Apart from the primary information, information related to function/activity of the peptides and proteins have also been compiled. Based on the role of mode of activity, therapeutic peptides and proteins are classified into four groups, Group I consist of the proteins with enzymatic or regulatory activity, Group II of those with specific targeting activity, Group III are protein vaccines, and Group IV consists of diagnostic agents. The proteins with enzymatic or regulatory activity are further sub-divided into three categories: (Ia) those replacing a deficient or abnormal protein, (Ib) those augmenting an existing pathway, and (Ic) those providing a novel function or activity. Therapeutics with specific targeting activity being sub-divided into two categories: (IIa) peptides interfering with a molecule or organism, and (IIb) peptides delivering other compounds or proteins. Similarly, group III contains protein vaccines, which are sub-classified as (IIia) vaccines, which protect against a deleterious foreign agent, (IIib) which treats autoimmune diseases and (IIic) treats cancer [29].

**Overview of the function/mode of activity-based classification of therapeutic proteins and peptides**

The current version of THPdb holds a total of 852 entries providing information on 239 US-FDA approved peptide and protein drugs and their 380 drug variants. Corresponding drug variants of therapeutic proteins and peptides were classified on the basis of a classification presented by Leader et al. of the 380 drug variants, 229 involved in regulatory and enzymatic activity followed by 78 belonging to the therapeutics with special targeting activity. A total of 58 drug variants belong to the vaccines category and 15 to the diagnostic agents.

In addition, these drug variants have also been grouped on the basis of disease in which they are being used for therapy. A total of 89 drug variants show activity in case of metabolic disorders, 80 have activity in the immunological disease area, 74 for hematological diseases, 61 in the cancer therapy, 63 in hormonal disorders, 46 variants useful for genetic disorders, 35 in infectious disease, 14 in cardiovascular disorders, 10 have the potential to cure bone disorders, 07 used in neurological disorder, 06 for respiratory disorder, 05 variants are given as adjunct, 03 in eye disorder and 01 variant has been used in malabsorption disorder. The routes by which these variants are being delivered have also been compiled systematically. Total 158 drugs by intravenous infusions, 116 drug variants are delivered by subcutaneous injections, 49 drugs by intramuscular route, 13 drugs via the oral route, 4 by intra-vitreal, 2 by intraresional, 2 by intratroacheal, 1 by intracoronary, and 3 drug variants are being used externally as ointments [30].

**CONCLUSION**

Improvements in peptide screening and computational biology will continue to support peptide drug discovery. Metabolomic,
proteomic, and genomic screening of toxins and other sources of natural products can identify bioactive peptides that may contain unique structural features generated by uncommon post-translational modifications or non-ribosomal synthesis. An improved understanding of the molecular basis for human genetic disorders can generate new potential therapeutic leads, and the de-improvement of poorly-characterized peptide receptors can stimulate research efforts for new receptor-ligand pairs [31].

Peptides have gained increased interest as therapeutics during recent years. More than 60 peptide drugs have reached the market for the benefit of patients and several hundreds of novel therapeutic peptides are in preclinical and clinical development. The key contributor to this success is the potent and specific, yet safe, mode of action of peptides. We believe that the future development of peptide drugs will continue to build upon the strengths of naturally occurring peptides, with the application of traditional rational design to improve their weaknesses, such as their chemical and physical properties. We also expect that emerging peptide technologies, including multifunctional peptides, cell penetrating peptides and peptide drug conjugates, will help broaden the applicability of peptides as therapeutics.

The enzymes in the GI tract are specifically designed to breakdown proteins and peptides into their amino acid counterparts resulting in the low oral bioavailability for the peptide drug that is observed. Researchers have devised and improved upon various methods to improve the oral bioavailability of peptide drugs including the use of penetration enhancers, enzymatic inhibitors, formulation approaches such as liposomes, nanoparticles, microemulsions, mucosal delivery, chemical modification approaches including peptide analogue design, peptide pro-drug design, cyclisation and pegylation have also been focused areas of research for the improvement of peptide drug oral bioavailability. Modest success in improving peptide oral bioavailability have been displayed by such approaches, however, only a very limited number of peptide drugs are in current clinical use in oral formulation forms. Among these approaches, the chemical modification seems to elicit the pathway to administer peptide drugs orally as this method can enhance both peptide drug enzymatic stability and permeability in vivo.

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AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

Declared none

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