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Peptides as 'Drugs': The Journey so Far

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Abstract Peptide based drugs are now a day fascinating application areas. This brief review focuses on the history of peptides, synthesis of peptides, their therapeutic applications and the current status of peptides in the market.

Keywords Peptides · Peptide drugs · Peptide history · Discovery of peptides · Synthesis of peptides

Introduction

Proteins and peptides are composed of short chains of amino acid monomers linked together via peptide bonds (–CONH–) and occur naturally in the human body (Yin et al. 2014). Peptides are attractive building blocks in bioorganic and supra-molecular chemistry because of the available diversity of amino acid sequences and their predictable conformational properties (Marine et al. 2015). Peptides are often classified according to the number of amino acid residues. Oligopeptides have 10 or fewer amino acids. Molecules consisting from 10 to 50 amino acids are called peptides. Peptides differ from protein in that the term protein describes molecules with more than 50 amino acids (http://www.peptideguide.com/).

Emil Fischer, along with Ernest Fourneau, synthesised the 'dipeptide'—glycylglycine in 1901. However, the discovery of peptide happened before that. In 1881, Theodor Curtius synthesized the first N-protected dipeptide, benzoylglycylglycine. The discovery of peptides was achieved by Curtius (Chandrudu et al. 2013). However, the work of Emil Fischer and his colleagues did stand out. That's why Emil Fischer is considered to be the founding father of the field of peptide chemistry and originator of the term 'peptide'. He was able to establish the type of bond that would connect them together in chains, namely, the 'peptide bond', and by means of this, he obtained the dipeptides and later the tripeptides and polypeptides (Grant 2002).

Subsequent to this brilliant beginning, progress however was slow for the next 50 years. In 1953, the chemical synthesis of the first polypeptide—'oxytocin' by du Vigneaud was a landmark achievement (Suresh Babu 2001). In 1963, Bruce Merrifield developed a rapid, simplified and effective way to prepare peptides and small proteins, i.e. the solid phase synthesis (SPPS). SPPS methodology has brought about a revolution in peptide and protein chemistry and thousands of different peptides have now been synthesized using this approach. In addition, this methodology has created new possibilities in the research fields of peptide-protein chemistry and nucleic acid chemistry It has greatly stimulated progress in biochemistry, molecular biology, pharmacology and medicine. It is also of great practical importance, both for the development of new drugs and for gene technology (Muheem et al. 2014).

The study of peptides and protein based biomaterials is of interest in itself because it probes and advances basic understanding of how natural biomaterials are constructed and used in biology (Woolfson 2010). Peptides regulate most physiological processes, acting at some sites as endocrine or paracrine signals and at others as neurotransmitters or growth factors (http://www.peptideguide.com/). They are very specific in activity when compared to small molecules when used as a drug candidate. As peptides are readily degraded inside the human body, they had

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been held ineligible for drug development in the past and deemed widely inferior to small molecules (Uhlig et al. 2014). The proteolytic stability of natural peptides is one of the principal limitations of their use as drug candidates (Vlieghe et al. 2010). Despite such neglect, a number of recent technological breakthroughs and advances have sparked major interest in their usage both as diagnostics as well as therapeutics (Uhlig et al. 2014). Peptides do not possess all the ideal qualities required for a drug, but properties such as high specificity, affinity and ability to stay in target areas longer due to their size and sometimes their ability of being easily degraded are very beneficial for many ailments. Recent trend suggests that these properties make peptide/protein drugs more popular than other drugs in certain diseases such as cancer, enzyme deficiency disorders, protein-dysfunction disorders, genetic and degenerative diseases or even in case of infectious diseases, where direct introduction of therapeutic proteins or peptides are desirable (Dulal 2010). Generally, peptides have a very few side effects and have become popular candidates for drug design (Yin et al. 2014). In the last three decades' therapeutic peptides and proteins have shown wide applications in medicine and biotechnology and have risen in prominence as potential drug of future (Ratnaparkhi et al. 2011).

Currently, there are sixty FDA approved peptide drugs in the market. About 140 peptide drugs are in clinical trials and over 500 are in pre-clinical development (http://www.peptideguide.com/). Thus, the peptide and protein based pharmaceuticals are rapidly becoming very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future.

History/Discovery of Peptide Drugs

Peptide synthetic techniques based on chemical methods have over 100 years of history which has been summarized in the scheme of events (Table 1). The first dipeptide, was discovered by Theodor Curtius. In 1881, Curtius synthesized the N-protected benzoylglycylglycine, where the silver salt of glycine was treated with benzoylchloride using the azide coupling method (Chandrudu et al. 2013). However, the term 'dipeptide' was firstly characterized by Hermann Emil Fischer (1902 Nobel Prize Laureate for Chemistry) on September 22, 1902 at Karlsbad during the 14th meeting of the German scientists and physicians. In 1901, Emil Fischer with Ernest Fourneau published an article which reported the preparation of the first dipeptide, glycylglycine, obtained by partial hydrolysis of the diketopiperazine of glycine in the laboratory (Fischer and Fourneau 1901; Suresh Babu 2001). This is considered as beginning of peptide chemistry.

However, both Curtius and Fischer faced difficulties. The peptides obtained by Curtius were those having blocked amino groups like acyl (acetyl or benzoyl). But unfortunately, at that stage, there was no means to remove the benzoyl residue from the nitrogen atom without destroying the peptide bond. Fischer didn't apply the 'azide' method of Curtius. His important contribution to peptide chemistry was the introduction of α-halogen fatty acid chlorides, in which the halogen atom, after coupling to an amino component, can be converted to an amino group by treatment with ammonia to yield a new amino acyl terminus. But the synthesis of enantiomerically pure peptides was still difficult. To overcome synthetic difficulties, there was a need to develop protecting groups that could protect amino part temporarily (Wieland 1955). The search for a mild amino-protecting group led to the discovery of benzyloxycarbonyl (also known as carbobenzoxy or Cbz or simply denoted by 'Z') group by Max Bergmann and Leonidas Zervas in 1932 (Gopi et al. 1999; Bergmann and Zervas 1932). The Cbz or Z group could be cleaved by catalytic hydrogenations under very mild conditions to yield toluene and N-carbonic acid which decomposes to CO_2 and the free aminoacid. This was a major breakthrough in peptide chemistry (Wieland 1955).

The acidolytic cleavability of Z group gave rise to elaboration of multitude of easily removable residues. The number of acid-labile protecting groups was very effectively enlarged by the discovery of *tert*-butyloxycarbonyl group (known as the Boc group) by L.A. Carpino, Mckay, and Albertson in 1957 (Wieland and Bodanszky 1991). An attractive approach to the formation of peptide bond was the use of 'coupling reagents'. In 1955, John Sheehan and George Hess introduced the most successful coupling reagent, 'dicyclohexyl carbodiimide' (DCC). Discovery of DCC along with other carbodiimides led to the efficient synthesis of peptides (Bodanszky 1984).

After the Second World War, the medicinal use of synthetic peptides started. This was the time of oxytocin and vasopressin, cyclic nanopeptides with one disulphide bridge and of the angiotensins. Vincent du Vigneaud isolated and characterized oxytocin and vasopressin from extracts of the posterior pituitary gland. In 1950, still peptide chemistry was emerging as a discipline. Though synthetic chemists developed methods to synthesize small peptides, their purification, but were not yet very efficient. Even sequencing of peptides had not yet been introduced. In fact, there was controversy as to whether each peptide and protein was a stoichiometric entity (i.e., had a unique and invariable sequence) or whether a given protein or peptide was composed of molecules each varying somewhat from the other but the average being fairly constant for that peptide. In the late 1950s, Fred Sanger devised a method for elucidating the amino acid sequence of a

Table 1 Scheme of events

S. No.	Year	Name of the discovery/invention
1	1881	Discovery of dipeptide
2	1901	First published synthesized dipeptide
3	1932	Discovery of the Z group
4	1953	Chemical synthesis of first polypeptide—oxytocin
5	1955	Introduction of the coupling reagents such as DCC
6	1957	Discovery of the Boc protecting group
7	1957–1958	Introduction of the column chromatographic purification of peptides
8	1963	Discovery of the SPPS
9	1970	Introduction of the Fmoc protecting group
10.	1970–1973	Discovery of other resins such as BHA resin and Wang resin
11	1976	Introduction of Prep HPLC method for purification of peptides synthesized by SPPS.
12	1990's	Development of native chemical ligation method for peptide synthesis
13	1999	Development of the large scale synthesis of Enfuvirtide (Fuzeon or T-20) by Trimeris-Roche

protein and applied it to insulin, and it was conceded that all proteins and peptides had exact molecular compositions. Column chromatography became generally available in the late fifties, and Edman introduced the manual procedure for sequencing peptides at about the same time. These procedures were used in the isolation and characterization of angiotensin and bradykinin. From the 1960's to the end of millennium, peptides were often considered as drugs of future (Loffet 2002). The introduction of the solid phase approach in 1963 by Robert Bruce Merrifield was a major breakthrough in history of peptide synthesis (Wieland 1955).

In the late sixties Roger Guillemin and Andrew Schally isolated TRH from hypothalamus, which was very costly and time consuming and required all the patience and skill of these two outstanding scientists. At about the same time many other biologically active factors that were apparently peptide in nature were also reported–substance P, many growth factors, including nerve growth factor, and a host of lymphokine activities including the interferons. Isolation and characterization of these had to await newer developments (Loffet 2002; Sidney 1988). The advent of molecular biology and advancement in synthetic methodologies and peptidomimetics, marked the beginning of a new era in peptide and protein therapeutics with the vision that there should be no limit to what can be produced as therapeutics (Fotouhi 2015).

Peptide Synthesis

Peptides can be synthesized mainly by two approaches—the Solution Phase Peptide synthetic approach and the Solid Phase Peptide synthetic approach.

Solution Phase Peptide Synthesis

It was first developed in 1920 by Emily Fischer who synthesised the first di-peptide—'glycylglycine' by this method. The procedure involved blocking the carboxyl group of one amino acid and the amino group of the second amino acid. Then, by activation of the free carboxyl group the peptide bond could be formed, and selective removal of the two protecting groups would lead to the free dipeptide (Nobel lecture and December 1984).

The search for mild protecting amino protecting group was a major target in early development of peptide synthesis. in orger to achieve that, Bergmann and Zervas replaced ethoxycarbonylchloride of Fischer by benzyl and thus created Benzyloxycarbonyl protecting group or the Cbz or Z group. The Z group was introduced into amino acids by reaction with benzyloxycarbonyl chloride (Wieland 1955).

Parallel to the discovery of new methods of protection, new procedures for the activation of the carboxyl group and novel methods for coupling have been numerous. If the methods of protection, activation and coupling, and deprotection were based on unequivocal chemistry, synthesis of peptides could be accomplished with perfection and resulting products would be single entities (Bodanszky 1984). However, it is a long and difficult task and synthesis of a single peptide takes around 1–2 years (Loffet 2002). The classical approach is not ideally suited to the synthesis of long chain polypeptides because the technical difficulties with solubility and purification become formidable as the number of amino acid residues increases (Merrifield 1963). The need to isolate and purify the intermediates is the major disadvantage of this approach (Carpino et al. 2003).

Solid Phase Peptide Synthesis (SPPS)

The solid phase peptide synthesis has been summarized in Fig. 1. In 1963, Bruce Merrifield introduced 'Solid phase peptide synthesis' (SPPS) and he was awarded Nobel Prize for the same. The concept of solid phase approach depends on the attachment of the first amino acid of the chain to a solid polymer by a 'covalent bond', the addition of the succeeding amino acids one at a time in a stepwise manner until the desired sequence is assembled, and finally the removal of the peptide from the solid support. The reason for this approach is that when the growing peptide chain is firmly attached to a completely insoluble solid particle, it is in a convenient form to be filtered and washed free of reagents and by-products. Thus the intermediate peptides can be purified, not by the usual recrystallization procedures, but by dissolving away the impurities (Merrifield 1963). This greatly simplified the manipulations and shortened the time required for the synthesis of the peptides. The major problem with this approach is handling the large amounts of expensive resins or large excesses of raw materials and reagents which increases the overall cost of manufacturing (Carpino et al. 2003).

With over hundred years of knowledge in peptide chemistry, cultivated with the two Nobel Prizes in the field—the discovery and solution phase synthesis of Oxytocin, the molecule of love, by Vincent du Vigneaud and the invention of solid phase peptide synthesis by Robert Bruce Merrifield, chemists are now able to prepare at will, essentially any desired peptide sequence (Brik 2013).

Alternative Synthetic Strategies

(1) *Combination of solid phase and liquid phase peptide synthesis (The 'hydrid' approach)* In this approach, the desired peptide sequence is constructed in the form of small protected fragments via solid phase synthesis using a resin solid support, after which fragments are cleaved from support, isolated, purified and characterized. Then, the fragments are assembled via solution phase coupling. In this way, the advantages of solid phase are combined with solution phase. This is particularly useful for the synthesis of very larger peptides. But multiple solution phase coupling steps are uncommon as they may affect solubility, purity and overall yield of the peptide because all these factors are affected as the length of peptide increases. But certain specific intermediate peptide fragments lend themselves particularly well to solution phase coupling steps and therefore are successfully synthesized by the 'Hybrid' approach. For example: Synthesis of Anti HIV drug—Enfuvirtide (Fuzeon or T-20). It is a

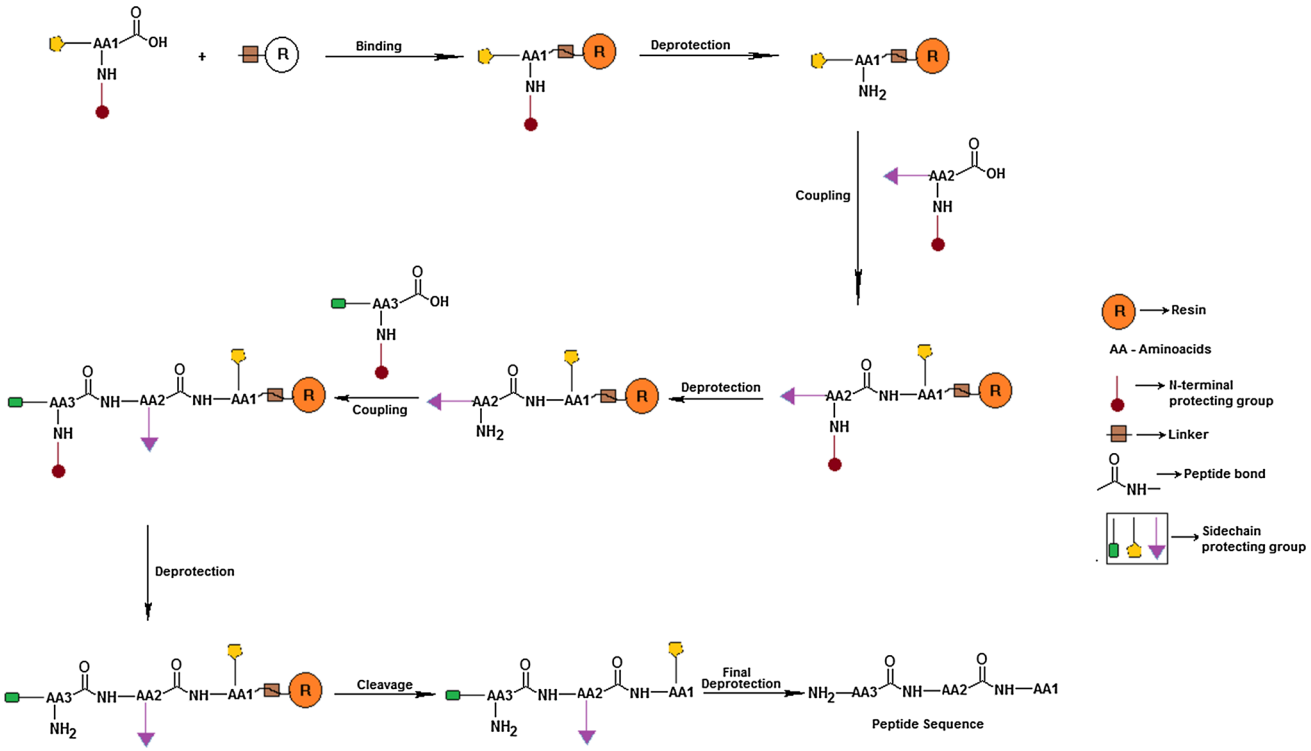

Fig. 1 Solid phase peptide synthesis technique

Table 2 Source FDA and drug bank website

S. No.	Generic name/name of API	Brand name/trade name	Therapeutic indications
1	Exenatide	Byetta	Type 2 diabetes. Preclinical Studies also revealed its neuroprotective role in Alzheimer's and Parkinson's disease
2	Nesiritide	Natrecor	Vasodilatory, natriuretic, diuretic and neurohormonal effects
3	Goserelin	Zoladex	Breast cancer and prostate cancer.
4	Ceruletide	Takus	Potent cholecystokinetic agent with a direct spasmogenic effect on the gallbladder muscle and bile ducts in humans and animals
5	Bentiromide	Chymex	Approved by FDA for screening test of exocrine pancreatic insufficiency in patients with cystic fibrosis.
6	Enfuvirtide	Fuzeon	A fusion inhibitor for the treatment of HIV infection.
7	Vancomycin	Alvanco, Vancocin	Treat colitis (inflammation of the intestine caused by certain bacteria) that may occur after antibiotic treatment.
8	Leuprolide	Eligard, Lupron, Lucrin, Lupride	Treatment of prostate and breast cancer, and to treat fibroids and endometriosis, Alzheimer disease
9	Oxytocin	Pitocin, Syntocinon	Used in labor induction augmentation of labor, post partum abbreviation of third stage of labor, post partum control of urinary bleeding, termination of pregnancy and for the evaluation of fetal respiratory capability.
10	Gramicidin	Neosporin ophthalmic, Ocu-spore-G	Used to treat skin lesions, surface wounds and eye infections.
11	Corticotropin	Acthrel, HP Acthar Gel	Allergies, ankylosing spondylitis, psoriasis, chorioditis, erythema, keratitis, sclerosis, dermatomyositis, rheumatoid arthritis, Stevens-Johnson Syndrome, Systemic Lupus Erythematosus, Ulcerative Colitis
12	Icatibant	Firazyr	Hereditary angioedema
13	Bivalirudin	Angiomax	Direct thrombin inhibitor
14	Gramicidin D	Neosporin	Antibiotic
15	Bacitracin	BACiiM	Antibiotic
16	Cetrorelix	Cetrotide	GnRH antagonist, used to treat hormone-sensitive cancers of the prostate and breast.
17	Pramlintide	Symlin	Diabetes
18	Teduglutide	Gattex, Revestive	Short bowel syndrome
19	Lepirudin*	Refludan	Anticoagulant
20	Daptomycin	Cubicin	Systemic infections caused by gram positive organisms
21	Octreotide	Sandostatin (Novartis)	Growth hormone producing tumors
22	Sincalide	Kinevac	Diagnostic aid to determine gall bladder and pancreas disorder
23	Aprotinin	Trasylol	Antifibrinolytic
24	Pasireotide	Signifor	Cushing's disease and Acromegaly
25	Telavancin	Vibativ	bactericidal
26	Dalbavancin	Dalvance	antibiotic
27	Teicoplanin	Targocid	Antibiotic
28	Oritavancin	Orbactiv	Antibiotic
29	Thymalfasin	Zadaxin	treatment of Hepatitis B and C
30	Histrelin acetate	Supprelin, Vantas	Treatment of prostate and breast cancer
31	Triptorelin pamoate	Trelstar	Treatment of prostate and breast cancer
32	Degarelix acetate	Firmagon	Treatment of prostate cancer
33	Ganirelix acetate	Ganirelix	As a GnRH antagonist, primarily used in assisted reproduction to control ovulation
34	Carbetocin	Duratocin, Deptocin	Carbetocin works as an oxytocic, antihemorrhagic and uterotonic drug in the peripheral nervous system
35	Tetracosactide	Synacthen, Cortrosyn	Analogue of adrenocorticotrophic hormone (ACTH) used for diagnostic purposes, treatment of adrenal insufficiency, different types of drug registant epilepsia
36	Teriparatide	Forteo	Teriparatide is the only anabolic (i.e., bone growing) agent indicated for use in postmenopausal women with osteoporosis
37	Eptifibatide	Integrilin	Angina, myocardial infarction

Table 2 continued

S. No.	Generic name/name of API	Brand name/trade name	Therapeutic indications
38	Bivalirudin	Angiomax	Angina
39	Saralasin acetate	Sarenin	Antihypertensive
40	Somatostatin acetate	Stilamin	Acute variceal bleeding
41	Calcitonin	Miacalcin, Fortical, Cibacal	Hypercalcemia, osteoporosis, pagets disease
42	Ecallantide	Kalbitor	Acute hereditary angioderma
43	Enfuvirtide	Fuzeon	AIDS
44	Glatiramer acetate	Copaxone, Glatopa	Used to treat multiple sclerosis
45	Atosiban acetate	Tractocile, Antocin	Delaying the birth in case of threat of premature birth
46	Lanreotide acetate	Somatuline Depot	Acromegaly
47	Vapreotide acetate	Octastatin, Sanvar	Treatment of esophageal variceal bleeding in patients with cirrhotic liver disease and AIDS-related diarrhea.
48	Ziconotide acetate	Prialt	Severe chronic pain as an analgesic
49	Nafarelin acetate	Synarel	Treatment of central precocious puberty, uterine fibriods and endometriosis
50	Peginesatide	Omontys, Hematide	Chronic kidney disease associated anemia
51	Desmopressin	DDAVP, Minirin	To treat nocturnal enuresis (bedwetting)
52	Lixisenatide	Lyxumia	Treatment of Diabetes
53	Ciclosporine (Cyclosporine)	Gangraf, Neoral, Sandimmune	An immunosuppressant drug widely used in organ transplantation to prevent rejection
54	Buserelin	Metrelef, Suprefact	Treatment of prostate and breast cancer
55	Gonadorelin (acetate and HCl)	Factrel, Lutrepulse Kit	Release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary

* Bayer has ceased the production of Lepirudin

36-aminoacid peptide, which acts as a fusion inhibitor. The large scale production of Enfuvirtide involves preparation of 2–3 intermediate peptide fragments via solid phase, subsequent purification of fragments and then, condensation of the fragments in solution phase (Nyfeler 1995; Han et al. 2006).

(2) *Convergent solid phase peptide synthesis (CSPPS)* Convergent peptide synthesis appears to be the most promising approach for the synthesis of long sequences of peptides. In this approach, protected peptide fragments are prepared by modifying the standard SPPS approach in such a way that instead of free peptide, protected fragments are cleaved from solid support which are then purified by column chromatographic techniques. The purified fragment is again bound to solid support resin, and the desired peptide sequence is constructed via steps involving multiple coupling cycles of protected fragments. This approach is often termed as Fragment Condensation Approach (Williams et al. 1997; Williams and Giralt 2000).

Overall, a convergent synthesis strategy for polypeptide synthesis can overcome many of the shortcomings related to classical solution phase synthesis using protected peptides. Importantly, convergent synthesis can avoid the accumulation of resin-bound impurities that otherwise limits stepwise solid phase peptide synthesis to chains containing approximately fifty amino acid residues (King 2010).

An alternate method of Convergent Synthesis is the Chemical Ligation. Native chemical ligation has overcome the limitations of the classical synthetic organic chemistry approach to the total synthesis of peptides, and enables the routine total or semi-synthesis of peptides. In this method, protected peptide fragments are first completely deprotected, and then these fragments are coupled by a chemoselective reaction to give a unique product with thio-ester linkage usually in aqueous solution.

The intermediate thio-ester then spontaneously rearranges to yield a full-length peptide with a native peptide bond at the ligation. Native chemical ligation

is an important step in peptide drug development (Dawson et al. 1994; https://en.wikipedia.org/wiki/Native_chemical_ligation).

Peptides as 'Drugs'/Peptides Based Therapeutics

Peptide drugs possess high specificity, affinity and they have ability to stay in target areas longer due to their size and sometimes their ability of being easily degraded are very beneficial for many ailments. These properties make peptide/protein drugs very popular (Dulal 2010). Higher ordered peptide structures with defined chemistry are capable of cellular targeting, recognition, and internalization (Branco et al. 2011). More than 7000 naturally occurring peptides have been identified, and these often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives (Fosgerau and Hoffmann 2015). The first recombinant protein therapeutic introduced was human insulin (Muheem et al. 2014). Various ailments that might be treated with this type of therapeutics include auto immune diseases, cancer, mental disorder, hypertension, certain cardiovascular and metabolic diseases enzyme deficiency disorders, protein-dysfunction disorders, genetic and degenerative diseases or even infectious diseases (Dulal 2010; Ratnaparkhi et al. 2011). Endocrine functions (especially diabetes mellitus and obesity), infectious diseases, and cancer are the major indications for use of peptide-based therapeutics. Whereas some peptide pharmaceuticals are drugs, acting as agonists or antagonists to directly treat cancer, others (including peptide diagnostics and tumour-targeting pharmaceuticals) use peptides to 'shuttle' a chemotherapeutic agent or a tracer to the tumour and allow sensitive imaging or targeted therapy (Sickert and Beck-Sickinger 2010). The clinical success of peptide based therapeutics has inspired the field of peptides into a wider horizon, with more than 130 different peptides or proteins already approved for clinical use by the FDA (Muheem et al. 2014).

A recent study also revealed that synthetic and naturally obtained human salivary peptides are active against breast, prostate, colon, osteosarcoma and bladder cancer. Costa et al. 2015 discovered antitumoral activity of human salivary peptides. Human natural salivary peptides, ranging from 5 to 500 μM were evaluated against two established tumor cell lines and it was revealed that there is a reduced cell population increase which is peptide-, cell- and possibly pathway-specific, with the most potent effect observed in observed in T-47D breast cancer cells (Costa et al. 2015). Peptide building blocks also find applications in biosensors, tissue engineering, and the development of antibacterial agents (Merrifield 1963).

Table 2 includes list of 55 peptide drugs approved by US FDA.

Peptides as 'Drugs'—Pros & Cons

Pros

- The most important advantage of peptide therapeutics over all small molecule drug candidates is the structural relationships between the constructed peptide and the physiologically active parent molecules from which they are derived, an attribute that help in depicting the risk of unforeseen side-reactions (Ali et al. 2013).
- Peptides being highly selective and efficacious, at the same time, they are relatively safe and well tolerated (Vlieghe et al. 2010).
- Peptides have the potential to penetrate further into tissues owing to their smaller size.
- Therapeutic peptides, even synthetic ones, are generally less immunogenic than recombinant proteins and antibodies
- Peptides are advantageous over small molecules in terms of being specific and affinity for the target.
- Peptides are advantageous over antibodies in terms of size (Duncan Patrick McGregor 2008; Fotouhi 2015).

Cons

A number of key issues have hampered use of peptides as drug candidates:

- One of the most difficult challenges for peptides is the need for effective and patient-friendly delivery technologies. Several companies are pursuing oral delivery technologies, but so far, these approaches have had only limited success. As one industry executive puts it, "your stomach can't tell the difference between a peptide and a piece of steak." Other delivery approaches include nasal and pulmonary, but one of the main problems with all of these techniques is the resulting need for higher dosages. Although these dosages are generally well tolerated, the ability to make them cost effectively has been a real problem.
- A second shortcoming of peptides is their low metabolic stability (Latham 1999).
- Peptides are limited to targets with known bioactive peptides and they exhibit lower potency (relative to antibodies)
- They also have a short half-life, owing to rapid renal clearance, and lack in vivo stability due to protease degradation.

- Peptides also suffer from having limited access to the intracellular space and can contain potential immunogenic sequences (random peptides) (Fotouhi 2015).

Approaches/Strategies for Effective Development of Peptides as Drugs

The major problem encountered with the use of peptide drug is related to its absorption after oral intake and its bioavailability. The primarily function of gastrointestinal tract is digestion and uptake of nutrients, electrolytes and fluids. It also protects humans against systemic invasion of harmful agents such as toxins, antigens and pathogens. To fulfill this latter task, several protective mechanisms exist in the gastrointestinal tract (Hamman et al. 2005). Now a day with the advancement of biotechnology and genetic engineering, the industry is capable of producing number of therapeutic peptides and proteins commercially (Jani et al. 2012). Significant progress has been made in the last few years to overcome disadvantages in peptide design such as short half-life, fast proteolytic cleavage, and low oral bioavailability.

These advances include:

Chemical modification A chemical modification of peptide and protein drugs improves their enzymatic stability and/or membrane penetration of peptides and proteins. It can also be used for minimizing immunogenicity. Protein modification can be done either by direct modification of exposed side-chain amino acid groups of proteins or through the carbohydrate part of glycoproteins and glycoenzymes (Kipnes et al. 2003).

These include:

(1) *PEGylation* The term PEGylation describes the modification of biological molecules by covalent conjugation with polyethylene glycol (PEG), a non-toxic, non-immunogenic polymer. PEGylation improves drug solubility, reduces proteolysis and decreases immunogenicity (Veronese and Mero 2008).
(2) Substitution of one more L-amino acid with D-amino acids. For e.g., Vasopressin and Desmopressin (Ram et al. 2003).
(3) Increasing the hydrophobicity of a peptide or protein by surface modification using lipophilic moieties i.e. lipidisation or multimerisation. For e.g., palmitoylation of insulin (Hashimoto et al. 1989).

Co-administration of an enzyme inhibitor such as thiomers and polyacrylates to inhibit the metabolism of peptides by enzymes such as proteases (Shaji and Patole 2008).

Introduction of peptidomimetic elements into the sequences; and innovative uptake strategies such as liposomal, capsule or subcutaneous formulations (Sickert and Beck-Sickinger 2010).

Use of absorption enhancers such as detergents, surfactants, bile salts, Ca^{2+} chelating agents, fatty acids, medium chain glycerides, acyl carnitine, alkanoyl cholines, N-acetylated α-amino acids, N-acetylated non-α-amino acids, chitosans, mucoadhesive polymers, and phospholipids (Aungst 2000; Lecluyse and Sutton 1997).

Formulation approaches that include administering the peptide drugs in the form of enteric coated emulsions, microspheres, liposomes and nanoparticles such as nanotubes, nanospheres, nanofibrils, nanotubes. e.g.: Encapsulation of insulin in nanospheres using phase inversion nanoencapsulation. The insulin released over a period of approximately 6 h, was shown to be orally active, and had 11.4 % of the efficacy of intraperitoneally delivered insulin (Shaji and Patole 2008; Carino et al. 2000).

Mucoadhesives Mucoadhesive polymers are able to adhere to the mucin layer on the mucosal epithelium and thus results in the increase of oral drug bioavailability of protein and peptide drugs. Mucoadhesive polymers include polyacrylic acid or cellulose derivatives. For example—carbopol, carboxymethyl cellulose, and hydroxy methyl cellulose (Ram et al. 2003; Shaji and Patole 2008).

Current Challenges

One of the challenges facing the manufacturers of both peptide drug substances and peptide drug products today is the inability of regulatory authorities on different continents to come up with a harmonized set of guidelines that define what level of peptide impurities can be present in peptide therapeutics. It is a challenge for two reasons. First, no-one in the business is totally sure what is expected of them, and—secondly—designing manufacturing processes to produce a higher quality product than is required adds significantly to the cost of goods, possibly to the point where an effective drug loses its economic viability (Lax 2010).

Peptide Drug Market—Current and Future Trends

The therapeutic peptides market emerged in the 1970s, when Novartis launched Lypressin, a Vasopressin analogue. In 2000 the total ethical pharmaceutical market was worth about $265 billion, with peptides and proteins, excluding vaccines, accounting for more than 10 %.

Approximately 30 peptides had reached the market in the period between 1970 and 2003 representing a $5.3 billion opportunity in 2003 (over 1.5 per cent of the $325 billion global pharmaceutical market). In 2004 only a modest part, 10 billion USD per year, of the total pharmaceutical market, valued at 270 billion USD per year, consisted of peptide drugs. Most of these peptide drugs, which are usually relative simple linear peptides or peptide like molecules were based on the native peptide of moderate size (maximal 40 aminoacids). A typical example is Zoladex which is an altered GnRH molecule that has superagonist activity. GnRH is a 10 amino acid neuropeptide that switches the fertility axis on. (Merrifield 1963) The market for synthetic therapeutic peptides rose from 5.3 billion USD in 2003 to 8 billion USD in 2005. Among the different classes of peptides, GNRH/LHRH agonists (Leuprorelin, Goserelin) account for almost 50 per cent of the peptide market. Other key commercialised peptides include Sandostatin (Somatostatin analogue, Novartis), Glatiramer (Immunomodulator peptide, Teva), Salmon-calcitonin (Miacalcin, Novartis) and Desmopressin (DDAVP, Ferring) (Pichereau and Allary 2005).

The number of new chemical entities (NCEs) had been almost stable for about 10 years, with around 35–40 each year, but the number of peptide and protein NCEs had been continuously increasing during these years. The most prominent peptide drug in approved in 2003 was Enfuvirtide, which blocks HIV entry into cellular CD4 and can be considered the turning point of investor attitude to biotech in general and to peptide drugs in particular (Otvos 2008).

While 10 peptides were approved between 2001 and 2010, the current decade has thus far witnessed the approval of six new peptides therapeutics-a remarkable yearly increase. The number of peptides in development is also steadily growing roughly doubling every decade and there are 400-600 peptides in preclinical studies. This is due to advances made in our understanding of peptide stability, peptide synthesis and formulation over the last three decades. Although the market share of peptide drugs is still relatively small (about 2 % of global market of all the drugs), the approval rate of peptide drugs is twice as fast as rate of small molecules, and the market is growing similarly at a rate that is twice the global drug market (Fotouhi 2015). In 2010, there were a total of 60 peptide drugs that had, at that point, been approved in the USA by the Food and Drug Administration (FDA) and their combined sales approached 13 billion USD. Among these, the multiple sclerosis therapy Copaxone and the hormone-related products Leuprolide, Octreotide, and Goserelin have annual sales of more than $1 billion each (Thayer 2011). There were also 140 peptide drugs in clinical trials, and over 500 in pre-clinical development (Badiani 2012). This excludes peptides, proteins and antibodies extracted from natural sources or produced by recombinant DNA technology, cell-free expression systems, transgenic animals and plants and enzyme technology (Vlieghe et al. 2010).

The year 2012 proved to be a remarkable one for the peptide therapeutics sector. The expansion of the commercial clinical pipeline of these drugs during the late 1990s and 2000s ultimately led to first marketing approvals in 2012 for six peptides, the most ever to receive approvals as new molecular entities in a single year. The year 2013, however, has seen mixed results. An approval to one of the peptides (i.e. lixisenatide) had been granted, but another peptide (i.e. peginesatide) approved in 2012 was withdrawn because of safety issues (Kaspar and Reichert 2013). At that point, it had been estimated that the peptide market sales will reach $ 12 billion in 2013 (Vlieghe et al. 2010). But according to a new market report published by Transparency Market Research "Peptide Therapeutics Market—Global Industry Analysis, Size, Share, Growth, Trends and Forecast 2014–2020," the global peptide therapeutics market had surpassed all predictions and was valued at USD 18.9 billion in 2013 and is estimated to reach USD 25 billion by 2018 at a CAGR of 2.8 % from 2014 to 2020 (Craik et al. 2013; Bruno et al. 2013). The biggest and most recent example of a 'blockbuster & top selling' novel peptide drug is Liraglutide (marketed as Victoza) whose annual sales solely reached over 2.6 billion USD in 2013. It acts as GLP-1 agonist (Glucagon-like) used for the treatment of type-2 diabetes mellitus (http://www.transparencymarketresearch.com/pressrelease/peptide-therapeutics-market.htm).

The paradigm shift in interest by the pharmaceutical industry toward proteins is exemplified by recent successes of recombinant proteins (especially monoclonal antibodies) as blockbuster therapeutics (Craik et al. 2013). This dramatic market increase is driven by both growing incidences of cardiovascular and metabolic diseases, and technological enhancements in peptide synthesis that include high-throughput approaches. Hybrid technology combining solid and liquid syntheses is also contributing to the growth of peptide therapeutics, as are novel modes of conjugation (http://www.pegsummit.com/Peptides-Conference/). At present there are over 100 approved peptide-based therapeutics on the market, with the majority being smaller than 20 amino acids (Bruno et al. 2013). One of the biggest opportunity areas in the Protein Therapeutics market will be in the field of Biogenerics, which is expected to create a multi-billion-dollar market in future (Ratnaparkhi et al. 2011).

All in all, the future of peptide drugs is more than just 'bright'.

In 2014–2015, US FDA approved 9 drugs some of which are peptides and are listed below in Table 3:

Table 3 Source FDA and company website)

S. No.	Peptide name	Brand name (Manufacturer)	FDA approval date	Therapeutic indication and mode of action
1	Metreleptin	Myalept (Astra Zeneca)	Feb. 25th 2014	Used in complications of leptin deficiency. Acts by Leptin receptor activation. (https://investor.lilly.com/releasedetail.cfm?releaseid=871658)
2	Albiglutide	Albiglutide Tanzeum GSK	April 15th 2014	For treatment of type2 Diabetes Acts as GLP-1 receptor agonist (http://www.drugbank.ca/drugs?utf8=%E2%9C%93&type=biotech&filter=true&withdrawn=1&filter=true)
3	Dulaglutide	Trulicity (Eli Lilly)	Sep. 18th 2014	For treatment of type 2 diabetes in adults. Acts as GLP-1 receptor agonist (http://www.astrazeneca-us.com/media/press-releases/Article/20140225-us-fda-approves-orphan-drug-myalept)
4	Insulin degludec	Tresiba (Novo Nordisk)	Sep.25th, 2015	For treatment of type-1 & type-2 diabetes in adults
5	Parathyroid hormone	Natpara (NPS Pharm.)	Jan. 23rd, 2015	For the control hypocalcemia in patients with hypoparathyroidism

Table 4 List of peptide drugs withdrawn

S. No.	Name of drug	Drug category	Therapeutic indication	Reason for withdrawl
1	Abarelix	Anti-testosterone agents/other hormone antagonists	For palliative treatment of advanced prostate cancer	Immediate onset of systemic allergic reactions, some resulting in hypotension and syncope, have occurred after administration of Abarelix
2	Alefacept	Dermatologic agents/ immunosuppressive agents/ selective immunosuppressants	Treatment of moderate to severe chronic plaque psoriasis	Withdrawn due to low business. Safety and risk studies were found ok
3	Aprotinin	Hemostatics / serine proteinase inhibitors /trypsin inhibitors	For prophylactic use to reduce perioperative blood loss and the need for blood transfusion in patients undergoing cardiopulmonary bypass	Increased risks of complications or death during surgeries, as compared with alternate medications
4	Drotrecogin alfa	Antisepsis	For reduction of mortality in patients with severe sepsis.	Withdrawn from the market after a major study indicated it was not effective in improving outcomes in patients with sepsis
5	OspA lipoprotein	Vaccines	For prophylactic treatment of lyme disease	Due to poor market performance, and economic concerns
6	Sermorelin	Hormone replacement agents as GH-RH analogue, as diagnostic agent for tests for pituitary function	For the treatment of dwarfism, prevention of HIV-induced weight loss	It is less effective than other HGH preparations. Also, its more expensive than rHGH also available
7	Somatrem	Somatropin and somatropin agonists	Growth Hormone analogue used for growth in children	Due to inefficiency in secretion of growth hormone and side effects such as production for antibodies
8	Urokinase	Thrombolytic Agents / Enzymes	Treatment of pulmonary embolism, coronary artery thrombosis, IV catheter clearance, and venous and arterial blood clots	Due to safety reasons. There were issues concerning the safe manufacturing of the drug 'Urokinase'
9	Fusafungine	Antibiotic	Treatment of nasal, throat and other respiratory infections	Risk of potentially fatal allergic reactions

Peptide drugs which have been withdrawn from the market—There are various peptide therapeutics which have been withdrawn from the market as summarized in Table 4 (https://www.gsk.com/en-gb/media/press-releases/2014/gsk-receives-us-approval-for-once-weekly-type-2-diabetes-treatment-tanzeum-albiglutide/).

Conclusion

Peptides are the class of biological molecules that both, offer and possess, a very wide range of chemical diversity. The importance given to peptide nowadays is not surprising. The amino acid sequence, whether in a peptide or a protein, is involved in all functions of our body at the cellular level. The specificity of the peptide and the potential it carries has already proven that it is highly effective as 'therapeutics'.

The Peptides are fast growing as the preferred choice of therapeutic drugs over the conventional drugs. More than 100 peptide drugs have reached the market, for the benefit of patients, including 6 peptides launched in the current year 2015 itself, the market for protein—peptide-based drugs is currently estimated at > $40 billion per year, or 10 % of the ethical pharmaceutical market. This market share is growing much faster than that of other pharmaceuticals, and success rates for bringing biologics to market are now about twice that of small molecule drugs. With several hundreds of novel therapeutic peptides already in preclinical and clinical development phases, the graph would only rise higher from here onwards. The potential of peptide drugs is enormous, and therefore, with recent advances in peptide science, the coming era could well be termed as 'The Peptide Era'.

Compliance with Ethical Standards

Conflict of Interest Sakshi Sachdeva declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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