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Review Article

Drugs Based on Bioactive Oligopeptides

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Abstract

Oligopeptides, i.e. biopolymers containing up to fifty amino acids, are being recognized as first-line treatments for a growing number of disorders. The review encompasses various aspects of the application of these active pharmaceutical ingredients, ranging from methods for obtaining a peptide molecule and formulating a dosage form, including excipients and their key properties, to various information on the pharmacokinetics and pharmacodynamics of peptide drugs supported by scientific experimental data, as well as modern quality control methods. The review considers that the application of peptide therapeutics covers a wide range of diseases. They include cancers of various genesis; bacterial infections; type 2 diabetes, neurological diseases, and eye diseases. The review notes that this is just a small fraction of the nosologies in which peptide bioregulators have demonstrated effective clinical activity. The review considers the role of excipients. A distinctive feature of the review is the consideration of innovative methods for quality control of peptide therapeutics. The methods include: high-performance liquid chromatography with tandem mass spectrometry, ultracentrifugation with flow-through rotors, dynamic laser light scattering, small-angle laser light scattering. The review specifically highlights the analysis of dispersion in turbid and opaque media – two-dimensional dynamic laser light scattering based on the kinetics of diffuse reflection with data analysis using a mathematical topological model. A non-invasive method for detecting intrinsic radiothermal emission of biologically active nanoparticles, which can be easily used for peptide molecules, is also described. The review presents a hypothesis according to which the background level of peptides forms a specific electromagnetic field of cells and tissues.

Keywords: peptide drugs, modern drugs, safe drugs, peptide drugs review, peptide synthesis, peptide pharmacokinetics, peptide pharmacodynamics, drug excipients.

Introduction

The production of peptide therapeutics is a classic example of green chemistry [1]. The biological origin and non-toxic degradability of these medicinal substances in combination with the recycling of blisters and paper packaging, as well as various types of "green" synthesis are modern vectors of the development of pharmacy[2]. Preclinical trials of oligopeptide molecules have shown that, due to their bio-origin, they have a safe action profile in tests on animals in terms of acute-chronic toxicity [3]. Peptide compositions daily demonstrate their effectiveness against cancers [4], show increased antibacterial activity in a number of studies [5–7], improve the permeability of cell membranes [8], and improve the pharmacokinetic parameters of medicinal substances [9]. Peptide compositions are widely used as a complex therapeutic in the treatment of many diseases [10], they significantly improve cognitive functions in the fight against Alzheimer's disease [11] and improve physiological parameters in ophthalmological therapy [12,13]. The following sections will describe the methods of peptide therapeutics production, their dosage forms available on the pharmaceutical market, the composition of finished therapeutics, the targets of the action of oligopeptides, and the pharmacokinetic parameters that contribute to this.

1. Production of Peptide Therapeutic Agents

Modern peptide molecules are obtained in two ways: artificial and natural [14,15]. The difference between these ways is that the natural method uses extracts from tissues, organs of animals and plants. [16,17], and artificial synthetic methods involve the synthesis of amino acid sequences *de novo* [18].

- Natural production method

The natural production method depends on the biological entity (animal tissue, plant material, cell culture, including recombinant cells [19,20].

The process flow for obtaining a peptide from biomaterials resembles the classical scheme of chemical-toxicological preparative purification and includes the following operations: Obtaining an organ/tissue/cell containing the peptide → Homogenization with the addition of a preservative to extend the shelf life of the homogenates → Purification of the homogenate by any

convenient method → Formation of extracts with different pH values. If the final objective is an extract, we shall stop at this step and proceed with purifying the extract by high-performance liquid chromatography. If the final objective is a specific oligo- or polypeptide, we shall move on to the next step → Purification of the extract → Centrifugation or a similar method for separating peptide molecules [21] → Purification of the peptide by any available chromatography [22] → Purity and authenticity analysis using a mass spectrometer [23]. Despite chromatographic purification, the resulting oligopeptide therapeutics still carry the threat of toxic impurities for plant extracts [24], HPV impurities (for extracts from cell cultures) [25], and prion impurities (for extracts from animal tissues, especially for brain extracts) [26–28].

- Artificial production method

The artificial method is quite diverse; a number of studies demonstrate that research teams in many countries are trying to move away from the use of animal-based oligopeptides to synthetic ones. [29].

The most popular method for creating oligo- and polypeptide chains is solid-phase synthesis with the protecting group Fmoc PG (Fluorenylmethyloxycarbonyl protecting group) [30].

The method consists in creating a solid-phase peptide resin (the most popular are: Merrifield resin [31], polyethylene glycol-grafted polystyrene (PEG-PS) [32], acrylamidopropyl polyethylene glycol (PEGA) [33]).

The solid-phase synthesis method is used for continuous creation of peptides[34], being a process amenable to automation[35] when the operator only needs to add Fmoc PG protected amino acids in the desired sequence. The principle of working with Merrifield resin [36]is as follows: Treatment of the resin with a Fmoc PG protected amino acid in an alkaline medium → removal of the Fmoc protection → addition of the second amino acid with Fmoc PG as an acylating agent to the first amino acid, which results in the formation of a peptide bond → removal of the protective group and addition of the amino acid with Fmoc PG again → and so on until the required peptide is assembled → cleavage of the resulting peptide from the resin at the end → purification by reversed-phase chromatography [37]. The following methods of artificial peptide synthesis are not the most common ones, but used: liquid-phase and recombinant. The most important difference between the liquid-phase and solid-phase peptide synthesis methods is that the growing sequence of amino acids is protected by reagents in the liquid phase [38].

2. Dosage Forms and Excipients of Peptide Therapeutic Agents

In this section, we will consider a wide range of dosage forms (DF) of peptide therapeutics and their excipients (ES). The dosage forms of peptides available on the pharmaceutical market of the Russian Federation are quite diverse: Injectable form (subcutaneous [39], intramuscular [40], intravenous [41], parabulbar [42]); Therapeutics for oral administration (tablets [43]); Therapeutics for external use (nasal form [44], ointments and creams [45]); Suppositories [46].

- Injectable form of peptide therapeutic agents

The most popular representatives of the injectable form are the oligopeptide semaglutide [47] and liraglutide [48] – an analogue of glucagon-like peptide-1 (GLP-1), and the peptide hormone insulin [49]. The finished dosage form is a pre-filled pen: for convenient dosing of the therapeutic and independent administration of the therapeutic. However, this selection of peptide therapeutics is not not exhaustive; the pharmaceutical market often finds a therapeutic of polypeptides obtained from the cerebral cortex of cattle in prescriptions of a neurologist [50], polypeptides from the retina of cattle in prescriptions of an ophthalmologist [51], polypeptides of the pineal gland of cattle in prescriptions of an obstetrician-gynecologist [52], VLP (virus like particles) vaccines in prescriptions of an epidemiologist and therapist [53] – all these therapeutics are lyophilisates, which are subsequently dissolved in water for injection or a 0.9% sodium chloride solution to create a finished dosage form. The main excipients and their properties are described in Table 1.

Table 1: Brief description of excipients of injectable forms of peptide therapeutics.

Name	Characteristics and application	Dosage form
Glycine	The simplest amino acid can be used both as an active substance and as an excipient. The literature describes the positive effect of the [oligo/polypeptide + glycine] complex on the pharmacokinetic parameters of the finished dosage form [54]	Lyophilisate ↓ Solution for injection
Disodium hydrogen phosphate dihydrate	Inorganic sodium salt used as a pH regulator, as a buffering agent	Solution for injection in pre-filled pens
Propylene glycol	Organic molecule used as a stabilizer and solvent when the amino acid sequence consists mainly of hydrophobic amino acids	Solution for injection in pre-filled pens
Phenol	The so-called carbolic acid, which serves as a preservative, helps prevent the growth of bacteria and contamination of solutions	Solution for injection in pre-filled pens

Hydrochloric acid	Inorganic acid used to correct pH	Solution for injection in pre-filled pens
Sodium hydroxide	Inorganic base used to correct pH	Solution for injection (pre-filled pens, ampoules)
Water for injection	Water corresponding to the article of the same name of the General Pharmacopoeia Monograph, used as a solvent	Solution for injection (pre-filled pens, ampoules)
Zinc chloride	Inorganic zinc salt used as a stabilizer for the hexameric form of the insulin molecule, which prevents its degradation during storage [55]	Solution for injection in pre-filled pens
Glycerol	Simple triol compound used as a stabilizer for protein molecules, as a component of a storage buffer, and as a solvent.	Solution for injection in pre-filled pens
Meta-Cresol (m-Cresol)	Organic aromatic compound used as a preservative in sterile dosage forms [56]	Solution for injection in pre-filled pens
Tromethamol (tromethamine)	Organic amine used as a buffering agent and as an alkalinizer.	Solution for injection in pre-filled pens
Polysorbate 20	Derived from sorbitol, used as a solvent, stabilizer and emulsifier	Solution for injection in pre-filled pens

- Oral therapeutics

The tablet form of peptide therapeutics is not very common due to the fact that biomolecules undergo hydrolysis when taken orally [57]. However, many research teams have addressed this problem, using various techniques to protect against peptidases. Some manufacturers chemically modify the molecule [58,59], others cyclize the peptide [60]. Different methods are presented in Section **3**, Pharmacokinetics and Pharmacodynamics of Peptide Therapeutic Agents.

An example of tablet forms on the Russian pharmaceutical market is the pentadecapeptide therapeutic used to treat infectious and inflammatory diseases of the mouth and throat – Gramicidin C [61]. Another example of a tablet form is glucosaminylmuramyl dipeptide (GMDP) used to treat chronic respiratory tract infections, acute and chronic purulent-inflammatory diseases of skin and soft tissues [62]. A brief description of excipients for the creation of peptide tablets that allow action under conditions of oral administration is given in Table 2.

Table 2: Excipients for making tablet dosage forms of peptides

Name	**Characteristics and application**
Colloidal dioxide	Anti-adhesive agent, reduces the sticking of mass to equipment in the course of tablet manufacture
Talc	Anti-adhesive agent and glidant, improves the flow properties of powders and granules
Acesulfame potassium	Sweetener, used to adjust taste
Mint flavoring	Organoleptic substance, imparts a pleasant taste and aroma
Sorbitol	Filler and moisturizer, adds volume, improves consistency
Magnesium stearate	Lubricant, reduces friction between particles [63], improves the tableting process
Lactose monohydrate	Filler, provides volume and mass of solid dosage forms
Sucrose	Filler and binder, provides strength to tablets
Potato starch	Filler and disintegrant, improves disintegration of tablets
Methylcellulose	Thickener and stabilizer, forms viscous solutions
Calcium stearate	Lubricant, reduces friction between particles

- Therapeutics for external, rectal, and vaginal use

Peptides have found wide application in skin care products [64] as agents stabilizing the condition of the skin [65], in addition, most therapeutics with oligopeptides and polypeptides are available in the form of nasal drops/sprays [66], for example, the heptamerous peptide complex (Met-Glu-His-Phe-Pro-Gly-Pro) is used to treat most pathological conditions caused by brain injuries and neurotic disorders of various origins [67]. The nasal form of another heptamerous peptide (Thr-Lys-Pro-Arg-Pro-Gly-Pro (diacetate)) has an anti-anxiety effect with antidepressant and antiasthenic action [68], and therapeutics with interferons of various origins help fight various infections [69].

Suppositories with peptides find their application in anti-infective therapy [70] and as a treatment agent for chronic prostatitis [71]. You can see the characteristics of the excipients of all listed dosage forms in Table 3.

Table 3: Excipients for making of nasal drops and suppositories based on peptides

Name	Characteristics and application	Dosage form
Methyl parahydroxybenzoate (nipagin)	Antimicrobial preservative, especially effective against bacteria and yeast [72]	Drops
Purified water	Universal solvent, does not contain impurities, used as a base for drops	All dosage forms
Edetate disodium dihydrate	Complexing agent, stabilizer, antioxidant	Drops
Sodium hydrogen phosphate dodecahydrate	pH regulator, buffering agent	Drops
Potassium dihydrogen phosphate	pH regulator, buffering agent	Drops
Povidone	Stabilizer, binder, solvent	Suppositories
Macrogol 4000	Variant of suppository base	Suppositories
Cocoa butter	Suppository base	Suppositories
Lanolin anhydrous	Emulsifier, base for suppositories and ointments, improves penetration of active substances	Suppositories, ointments
Solid fat	Suppository base	Suppositories, ointments

3. Pharmacokinetics and Pharmacodynamics of Peptide Therapeutic Agents

Peptide therapeutic agents have a potential to be used in all areas of evidence-based medicine. The ability to synthesize different amino acid sequences allows for variation in the pharmacokinetics and pharmacodynamics of finished dosage forms [73]. This section will use scientific data to demonstrate the positive results that scientists have achieved to date and will also touch upon the future of peptide therapeutics.

In most instructions for peptide injection forms of therapeutics, you can find the phrase: "the therapeutic consists of a balanced and stable peptide mixture with polyfunctional activity, which does not allow the pharmacokinetic analysis of individual components". It corresponds to the logical conclusion about the specificity of administration, however other dosage forms allow measuring pharmacokinetic parameters, for example: the bioavailability of GMDP tablets is 7-13%. This bioavailability indicator allows research teams to propose solutions that are not suitable for most drugs, for example, a research team from China has synthesized a cyclic peptide using the method of through cyclization with disulfide bridges, as a result they managed to increase the stability and antibacterial activity of the molecule, as well as increase its half-life [74]. Italian scientists propose several methods for creating cross-linked peptides, which are basically arranged in an □-helix, using cross-linking agents (obtained by connecting the side chains of suitable modified amino acids located at the required distance within the peptide chain) to improve a number of properties of the active substance [75]. Other researchers propose a physical option to fight against poor pharmacokinetics, such as using a self-orienting swallowable device consisting of a stainless-steel core and low-density polycaprolactone that autonomously releases peptide-containing microneedles into the gastric epithelium [76].

A huge area that is gaining momentum every year is the use of peptides in various forms: 1) as an independent nanoparticle (NP); 2) as a complex of peptide + nanoparticle. This step allows improving the biological activity of the molecule – a pharmaceutical agent [77], allows selectively choosing a target for the delivered therapeutic [78] and peptide [79]; a variation in the preparation of a peptide-equipped nanoparticle, encapsulation of the peptide in liposomal nanoparticles [80], or in polymeric NPs [81], allows increasing the bioavailability of the peptide agent several times. At the moment, there are studies where insulin is enclosed in liposomal nanoparticles to protect the polypeptide from oral degradation and ensure the ease of drug administration; many studies are aimed at using metal

nanoparticles with peptides as antibacterial activity boosters [82] and as contrast agents [83].

4. Innovative Quality Control of Peptide Therapeutics

The qualitative and quantitative approach to the quality control of peptide therapeutics includes many instrumental methods of analysis:

- Chromatographic methods: high-performance liquid chromatography with tandem mass spectrometry[84]; reversed phase high performance liquid chromatography[85]; ion-exchange chromatography[86]; thin-layer chromatography[87]; affinity (biospecific) chromatography[88]; adsorption chromatography[89]; group-specific chromatography[90]; metal-chelate affinity chromatography [91]; covalent thiol-sepharose chromatography[92]; reversed phase chromatography[93], as well as gel filtration as a type of sieve chromatography[94].
- Electrophoretic methods: polyacrylamide gel electrophoresis (gel electrophoresis) [95]; moving-boundary electrophoresis (frontal electrophoresis) [96]; disk electrophoresis [97]; isotachophoresis [98]; isoelectric focusing [99]; two-dimensional gel electrophoresis [100]; pulsed-field gel electrophoresis [101].
- Centrifugation methods: analytical ultracentrifugation [102]; zonal high-speed and isopycnic centrifugation [103].
- Spectral methods: mass spectrometry[104]; dynamic laser light scattering [105]; small-angle laser light scattering [106]; dispersion analysis in turbid and opaque media – two-dimensional dynamic laser light scattering [107]; spectroscopy in the ultraviolet (UV)[108], visible and infrared (IR) bands [109].

The diversity of approaches to biomolecule analysis plays a key role in the comprehensive study of its characteristics used in quality control. But all these methods have one big drawback – a destructive approach to the studied object. Thus, creation of a non-invasive quality control method is a priority for pharmacists. For example, researchers from RUDN University, Moscow, have managed to implement a new non-invasive method for detecting inherent radiothermal emission of biologically active nanoparticles [110–114], which is easily applicable to peptide molecules. Analyzing the literature on peptide therapeutics, we can come to the conclusion that the mechanism of action of peptides is not always clear to analysts due to the fast biotransformation of peptide molecules; nevertheless, it is effective and clinical medicine proves it; of course, this does not apply to those peptide therapeutics that are structural analogues of various hormones and other biostructures, for which the mechanism of action has been thoroughly studied, but for most active peptide molecules it is still unknown. It allows a hypothesis that peptide bioregulators are included in a number of physicochemical characteristics of the cell (temperature, pressure, osmotic pressure, pH, dynamism, selective permeability) – electromagnetic fields of the cell. Considering the fact that this emission is possible only for nanoparticles, and most peptides are such, the pieces of the possible puzzle of regulation of the internal environment of the cell by peptides fall into place. Perhaps their property of radiothermal emission allows stabilizing the electromagnetic fields of all cells, because it is not uncommon for a peptide therapeutic to be effective where a classic therapeutic has already stopped working. Perhaps this hypothesis is the answer to the question about the mechanism of action of oligo- and polypeptides. In the future, further study of peptides is planned, both from the pharmaceutical point of view with approaches to quality control of peptide therapeutic agents, and from the biochemical point of view to clarify their mechanism of action.

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Contributors: All authors have read and approved the final manuscript.

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