Nanoparticles Functionalized with Venom-Derived Peptides and Toxins for Pharmaceutical Applications

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Abstract: Venom-derived peptides display diverse biological and pharmacological activities, making them useful in drug discovery platforms and for a wide range of applications in medicine and pharmaceutical biotechnology. Due to their target specificities, venom peptides have the potential to be developed into biopharmaceuticals to treat various health conditions such as diabetes mellitus, hypertension, and chronic pain. Despite the high potential for drug development, several limitations preclude the direct use of peptides as therapeutics and hamper the process of converting venom peptides into pharmaceuticals. These limitations include, for instance, chemical instability, poor oral absorption, short half-life, and off-target cytotoxicity. One strategy to overcome these disadvantages relies on the formulation of bioactive peptides with nanocarriers. A range of biocompatible materials are now available that can serve as nanocarriers and can improve the bioavailability of therapeutic and venom-derived peptides for clinical and diagnostic application. Examples of isolated venom peptides and crude animal venoms that have been encapsulated and formulated with different types of nanomaterials with promising results are increasingly reported. Based on the current data, a wealth of information can be collected regarding the utilization of nanocarriers to encapsulate venom peptides and render them bioavailable for pharmaceutical use. Overall, nanomaterials arise as essential components in the preparation of biopharmaceuticals that are based on biological and pharmacological active venom-derived peptides.

Keywords: Venom-derived peptides, therapeutic peptides, biopharmaceuticals, nanotechnology, drug delivery system, toxins.

1. INTRODUCTION

Venomous animals are specialized predators that have developed over millions of years of evolution, the ability to produce venoms, and secretions composed of an incredible structural and functional diversity of toxins for their biological purposes [1]. These compounds have evolved to deter, immobilize or kill a range of prey, predators, or competitors, in which the toxins can affect essential and vulnerable physiological processes of the affected organism, such as neuromuscular signaling, hemostasis, and cardiovascular function, among others [2]. When injected into animals or human victims, usually in small amounts, these compounds can cause harmful disorders and even death. Venoms are composed of complex mixtures of salts, low molecular weight organic molecules such as polyamines, amino acids, venom enzymes, and, importantly, pharmacologically active peptides and polypeptides. A wide diversity of animals has developed the ability to produce these cocktails of compounds, such as snakes, lizards, scorpions, spiders, centipedes, ticks, cone snails, sea anemones, jellyfish, fish, cephalopods, echinoderms, several insect orders (such as ants, bees, wasps), and even some mammals (the platypus, shrews, vampire bats) [3]. Venoms are delivered to prey or predators through diverse types of specialized structures such as barbs, beaks, fangs, or modified teeth, harpoons, nematocysts, pinchers, proboscises, spines, sprays, spurs, and stingers [4]. Some of these animals can produce venoms in special glands, while others may contain toxic substances that spread throughout their body tissues [5].

Over time, the biological diversity and potency of venoms have become more refined, so that they display high specificity to their pharmacologically active targets, rendering animal toxin compounds extremely valuable for the development of new therapeutics and adjuvant strategies in clinics [6, 7]. Venom-derived peptides act with excellent selectivity and potency on several biological targets, including ligand- and voltage-dependent ion channels, G-protein coupled receptors, membrane transporters, and enzymes. When used in a suitable dosage, these peptides may be useful therapeutics [8, 9]. In the pharmaceutical field, peptide toxins can be used in their original form (natural or synthetic) or
a chemically modified form, mimicking the desired action of the original peptide [2].

In this context, drugs derived from venom peptides or protein toxins were approved by the Food and Drug Administration (FDA, U.S. Department of Health and Human Services) for treatment of diverse clinical conditions, including diabetes, hypertension, and chronic pain. Several venom-derived peptides are also in preclinical development, while others are under clinical studies that have the potential to treat a range of diseases, including cancer and autoimmune diseases [10]. Examples of venom-derived peptides and pharmaceuticals prepared from venom-peptide leads approved by the FDA that are in current clinical use are summarized in Table 1. Although a relatively high number of peptides and proteins have been isolated from animal venoms, such numbers reflect only a small part of the huge chemical diversity found in marine and venomous terrestrial organisms. It has been argued that there exists a practically infinite potentiality to be explored in the field of venom peptides and derivatives [9, 10].

Considering that many current therapies are deficient, the importance of animal venoms as innovative resources for drug discovery becomes clear when noting therapeutic peptides are highly selective pharmacological agents. Despite the exceptional potential for drug discovery and development, several limitations have been reported for this class of compounds, hampering the process of converting venom peptides into therapeutic agents. The low stability, short half-life, and poor oral bioavailability are some of these barriers. Toxicity can often also be a limiting factor, capable of restricting the development of new drugs. A promising strategy that has been developed to overcome these drawbacks is the use of nanocarriers for delivery and improve the bioavailability of venom peptides [11, 12]. Previous reports have emphasized the importance of nanostructures to deliver toxins and animal venoms for diverse biological applications [13, 14]. Herein, examples of this technological approach are presented and discussed, from which information can be gleaned to select materials and conditions to encapsulate venom-derived peptides for pharmaceutical applications. In this scenario, the use of nanotechnology appears to be an advanced methodology that promises to overcome the intrinsic limitations of the bioactive venom-derived peptides in the development of biopharmaceuticals.

2. LIMITATIONS AND CHALLENGES OF PEPTIDES FOR PHARMACEUTICAL APPLICATIONS

Despite the potentially useful pharmacological properties of peptide toxins, their therapeutic application is often hampered by inconvenient aspects inherent to their structures. Similar to other peptide drugs, venom peptides generally exhibit varying degrees of chemical instability in vivo, since they have poor intestinal absorption, and are rapidly eliminated when they reach the bloodstream. In addition, the toxicity associated with venom peptides has also contributed to restricting the development of new drugs [15-17].

The intestinal uptake of peptides is generally limited by their high molecular weights and polarities, in a way that such characteristics, in this case, negatively interfere with the ability of these compounds to cross the intestinal membranes in an unmodified and active form. Moreover, the presence of proteases in the digestive system rapidly causes the amide bonds of peptides to break down, contributing to their low stability in vivo. All these facts preclude the oral administration of peptides, which is the most favorable route acceptable by patients, in detriment to much less convenient invasive alternatives [18]. Even if peptides can reach the bloodstream in certain cases, they are degraded rapidly by various circulating proteases, or even eliminated from the body by the hepatic and renal systems. This chemical instability is related to the individual structure of each peptide, such as stabilization by post-translational modification. Linear peptides (without disulfide bonds or other covalent constraints) are more susceptible to proteolysis and more rapidly degraded in vivo [17]. These characteristics could decrease the half-life of peptides in the bloodstream and, consequently, require repetitive administration to obtain their therapeutic effects when in use. Repetitive drug administration is an undesirable regimen for patients and contributes to decreased compliance with a given treatment [19]. Apart from these barriers, toxicity may also be associated with the use of venom peptides as pharmaceuticals, especially the antimicrobial and cytolytic peptides. These classes of peptides, expressed in the venom and venom glands of snakes, scorpions, bees, wasps and other animals can display an off-target effect causing cytotoxicity and damage to diverse biological systems [17, 20-24].

High toxicity to humans imposes, in some circumstances, the discontinuation of venom peptides in clinical trials of drug development platforms [25].

Considering these facts, some strategies have been devised to overcome these drawbacks, to advance the development of venom peptides for therapeutic purposes. Cyclization, for example, is a technique that has been tested to increase the resistance of peptides to proteolysis. Alternatively, PEGylation and covalent binding to larger proteins, as well as the incorporation of peptides into slow release polymer matrices have been studied to increase peptide half-life in the bloodstream [9]. Additionally, to increase peptide half-life, platforms for controlled release with nanocarriers that can be optimized to deliver the peptides and circumvent peptide structural susceptibilities and the intrinsic toxic side-effects have emerged as a promising strategy for the development of peptides as pharmaceuticals [26]. Fig. (1) schematically illustrates the main points involved in the development of biopharmaceuticals from venom peptides with the application of nanocarriers and nanotechnology.

3. THERAPEUTIC APPLICATIONS OF VENOM-DERIVED PEPTIDES

Venom-derived peptides have a range of biological activities useful for treating various clinical conditions, and some are already in clinical use (Table 1). Apart from these approved drugs, listed in Table 1, studies have shown for instance, venom bioactive peptides with antitumor effects are attractive compounds for the treatment of cancer. As examples, peptides TsAP-1 and TsAP-2 from the venom of the Brazilian yellow scorpion Tityus serrulatus showed an antiproliferative activity against human cancer cell lines [23]. Lycosin-1 and latarcin-2a, derived from spider venoms [27, 28], and the recently characterized vipericidin peptides [20], from rattlesnake venom glands, as well as crotamine and...
Table 1. Approved drugs from animal venom-derived peptides and leads that are in current clinical use.

<table>
<thead>
<tr>
<th>FDA* Approval</th>
<th>Drug Name (Active Ingredient)</th>
<th>Source (Species Origin)</th>
<th>Mechanism of Action</th>
<th>Indication</th>
<th>Dosage Form/Route</th>
<th>Dosage and Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1981</td>
<td>Captopril</td>
<td>Venom of the snake <em>Bothrops jararaca</em></td>
<td>Angiotensin-converting enzyme inhibitor.</td>
<td>Hypertension, Heart Failure</td>
<td>Tablet/ oral</td>
<td>25 mg to 150 mg two or three times a day.</td>
</tr>
<tr>
<td>1998</td>
<td>AGGRASTAT® (Tirofiban)</td>
<td>Venom of the snake <em>Echis carinatus</em></td>
<td>An antagonist of the platelet glycoprotein (GP) IIb/IIIa receptor, inhibits platelet aggregation.</td>
<td>Acute coronary syndrome</td>
<td>Injectable/ Intravenous</td>
<td>Initial rate of 0.4 µg/kg/min for 30 minutes and then continued at 0.1 µg/kg/min.</td>
</tr>
<tr>
<td>1998</td>
<td>INTEGRILIN™ (Epitifibatide)</td>
<td>Venom of the snake <em>Sistrurus miliarius barbouri</em></td>
<td>Prevents binding of fibrinogen, von Willebrand factor, and other adhesive ligands to GPIIb/IIIa receptors; inhibits platelet aggregation.</td>
<td>Acute coronary syndrome</td>
<td>Injectable/ intravenous</td>
<td>An intravenous bolus dose of 180 µg/kg as soon as possible following diagnosis, followed by a continuous infusion of 2 µg/kg/min until hospital discharge or the initiation of CABG surgery, up to 72 hours.</td>
</tr>
<tr>
<td>2000</td>
<td>ANGIOMAX™ (Bivalirudin)</td>
<td>Saliva of the medicinal leech <em>Hirudo medicinalis</em></td>
<td>A reversible direct thrombin inhibitor exhibits anticoagulant effects.</td>
<td>An anticoagulant in percutaneous coronary intervention</td>
<td>Injectable/ Intravenous</td>
<td>An intravenous bolus dose of 1.0 mg/kg followed by a 4-hour infusion at a rate of 2.5 mg/kg/h. If needed, an additional infusion may be initiated at a rate of 0.2 mg/kg/h for up to 20 hours.</td>
</tr>
<tr>
<td>2004</td>
<td>PRIALT® (rizonotide)</td>
<td>Venom of the cone snail <em>Conus magus</em></td>
<td>N-type calcium channel (Ca,2.2) antagonist; blocks excitatory neurotransmitter release in primary afferent nerve terminals and antino- ciception.</td>
<td>Severe chronic pain</td>
<td>Injectable/ Intrathecal</td>
<td>Initiated at no more than 2.4 µg/day (0.1 µg/h) and titrated to patient response. Doses may be titrated upward by up to 2.4 mcg/day (0.1 µg/h) at intervals of no more than 2-3 times per week, up to a recommended maximum of 19.2 µg/day (0.8 µg/h) by Day 21.</td>
</tr>
<tr>
<td>2005</td>
<td>BYETTA™ (Exenatide)</td>
<td>Venom of the Gila monster <em>Heloderma suspectum</em></td>
<td>An incretin-mimetic agent enhances glucose-dependent insulin secretion and several other anti-hyperglycemic actions of incretins.</td>
<td>Type 2 diabetes mellitus</td>
<td>Injectable/ subcutaneous</td>
<td>Initiated at 5 µg per dose twice daily at any time within 60- minutes before morning and evening meals. Based on clinical responses, the dose can be increased to 10 µg twice daily after 1 month of therapy.</td>
</tr>
</tbody>
</table>

*U.S. Food and Drug Administration, Department of Health and Human Services (http://www.fda.gov).

crotamine-derivatives [29, 30], have shown promising antimicrobial activity and potential to target and treat glioma tumors [31]. Cytotoxic activity against certain types of cancer cells is also exhibited by bee and wasp venoms, from which the peptides melittin and mastoparan were isolated, respectively [24, 32]. Melittin has been extensively studied as an antitumor agent, in addition to its antibacterial, antiviral, antifungal, and antiparasitic effects. Such biological activities are due to membrane destabilization by the peptide, forming pores or acting as a surfactant, causing membrane disruption. Thus, cell permeability is increased, and cell lysis invariably occurs [33, 34]. These membrane effects are common to other venom peptides and are potentiated in cancer cells because of greater electrostatic interactions due to the small size and net positive charge of these compounds [35].

Perturbation and disruption of the cytoplasmic membranes are also the main mechanisms of antimicrobial peptides that can be found in diverse animal venoms. These compounds have attracted special attention as a source of new anti-infective agents, especially with the emergence of drug resistance of microorganisms to conventional therapies. Such peptides are naturally present as part of the innate or adaptive immunity of animals and may cause destabilization of membranes and cell lysis, as well as interaction with the intracellular components, like DNA, contributing to the mechanism of toxicity in the targeted microorganism. Several antimicrobial peptides have already been characterized from the venom of bees, ants, scorpions, cone snails, snakes, spiders, centipedes, and wasps, and collectively a library with hundreds of active compounds against bacteria, fungi, protozoa, and viruses is available. These venom-derived antimicrobial peptides have considerable potency at minimal inhibitory concentrations (in the micromolar range). A limiting
Nanocarriers and nanotechnology to improve the bioavailability and delivery of venom-derived peptides. Venom peptides can be developed into target-specific drugs by circumventing some intrinsic drawbacks of peptides used as therapeutics, such as chemical instability, poor intestinal absorption, toxicity, and short half-lives. The improvement of these peptide limitations to turn peptides into biopharmaceuticals can be achieved by employing nanocarriers. (A higher resolution/colour version of this figure is available in the electronic copy of the article).

Fig. (1). Nanocarriers and nanotechnology to improve the bioavailability and delivery of venom-derived peptides. Venom peptides can be developed into target-specific drugs by circumventing some intrinsic drawbacks of peptides used as therapeutics, such as chemical instability, poor intestinal absorption, toxicity, and short half-lives. The improvement of these peptide limitations to turn peptides into biopharmaceuticals can be achieved by employing nanocarriers. (A higher resolution/colour version of this figure is available in the electronic copy of the article).

factor for many of these peptides is the recurrent nonspecific action on non-target cells and tissues, and a variable level of hemolytic activity, requiring additional strategies to render them viable for antimicrobial therapy [3, 16].

Venom peptides have been discovered and developed as therapeutic tools for diabetes mellitus [36]. Some of these peptides may act by mimicking endogenous metabolic hormones such as glucagon-like peptide-1 (GLP-1), stimulating the release of glucose. This is the case for exendin-4, derived from the Gila monster Heloderma suspectum, which is currently on the market in its synthetic form Exenatide (Byetta ™) for treatment of type 2 diabetes mellitus. Other GLP-1 analogs have recently been reported in monotremes species including the platypus Ornithorhynchus anatinus and four species of echidna. Another peptide, an analog of human insulin (ConIns G1), was found in the venom of the cone snail Conus geographus and showed significant agonist activity at the human insulin receptor with rapid release of insulin, but with a lower potency compared to insulin. Peptides can also act on ion channels expressed in pancreatic beta cells stimulating insulin secretion. Hanatoxin (κ-theraphotoxin-GR1A) from the venom of the tarantula Grammostola rosea and guangxitoxin-1 (κ-theraphotoxin-Pg1a) from the tarantula Chilobrachys guangxiensis may act as voltage-gated potassium-ion channel (subtype 2.1, KV2.1) blockers. The peptide Conkunitzin-S1, from the venom of the cone snail Conus striatus, can act as a blocker of the KV1.7 channel. Iberiotoxin (IbTx), isolated from the venom of the East Indian red scorpion Buthus tamulus, blocks BK channels. Despite their beneficial effects, some of these channels could be present in other cells and tissues of the human body, limiting the use of these peptides in therapy [36].

Other classes of peptides, derived from animal venoms, include examples with specificity for the cardiovascular system. For example, bradykinin-potentiating peptides (BPPs) potentiate the antihypertensive effects of bioactive kinins, inhibiting their degradation by angiotensin-converting enzyme (ACE) in the bloodstream. BPPs were originally found in the venom of the Brazilian pit viper Bothrops jararaca [37]. In this group, teprotide (Bj-PRO-9a) was the most promising peptide, and its most effective analog, captopril, was approved in the 1980s for the treatment of hypertension and congestive heart failure (Table 1). This class of peptides has also been found in the venoms of other species of snakes, such as Crotalus and Lachesis, as well as in the venom of other animals such as scorpions and spiders. Besides BPPs, natriuretic peptide (DNP-CNP Bj, TNP-a, b-TNP, TNP-c TsNP), antiarrhythmic peptides (Jingzhaoxin -I, PhKv, GsMTx -4), peptides similar to vasopressin (conopressins), and the selective inhibitors of the α1 adrenergic receptor (α-TIA conopeptides) from sea snails of the genus Conus are examples of other relevant animal venom toxins that have potential for the regulation of blood pressure and other cardiovascular disorders [38].
With regard to the cardiovascular system, blood hemostasis is another condition that can be affected and modulated by animal toxins, especially those derived from snakes. Interfering with platelet function, these compounds can act by several mechanisms involving disintegrins, C-type lectin-like toxins, three-finger toxins, phospholipase A2, serine proteinases, metallocproteinases, nucleotidases and L-amino oxidases [39]. The disintegrins block platelet aggregation by binding to the αIIbβ3 receptor, preventing the binding of fibrinogen. Despite disturbing hemostasis, peptides in this class of venom toxins were utilized for the development of two therapeutic and commercially available antiplatelet agents, tirofiban (Aggrastat™), based on an isolated venom polypeptide and eptifibatide (Integrillin™), a cyclic heptapeptide analog derived from the venom of Barbour’s pygmy rattlesnake Sistrurus miliarius barbouri [40, 41]. Other venom peptide components may act as antagonists of thrombin, a blood clotting enzyme responsible for cleaving fibrinogen into fibrin and leading to the formation of a blood clot. This is the case of bivalirudin (Hirulog™), a linear 20-residue peptide approved in 2009 for use as an anticoagulant drug. Bivalirudin was derived from hirudin, a mini-protein found in the salvia of the medical leech Hirudo medicinalis [15].

Animal toxins have also been shown to be active on pain receptors, especially ion channels such as sodium and calcium, among others. A variety of animals, including bees, wasps, spiders, ants, centipedes, scorpions, snakes, and cone snails have neurotoxin peptides that act on ion channels and may be potential candidates for the development of new analgesic drugs. Ziconotide (Prialt) was approved over a decade ago by the FDA for the treatment of chronic pain through the blockade of N-type calcium channels. This drug is the ω-conotoxin MVIIA, a synthetic peptide derived from the venom of the cone snail Conus magus [42, 43].

These few examples, summarized in Table 1, give a glimpse of the potential of venom-derived peptides for clinical and diagnostic applications. Further information regarding the research and development of toxins as biopharmaceuticals can be found elsewhere [44, 45].

4. NANOPARTICLES FORMULATED FOR DELIVERY OF VENOM-DERIVED PEPTIDES

4.1. Overview

The incorporation of drugs, and particularly, active peptides into nanoparticles can potentially solve several problems concerning the delivery of therapeutic agents in conventional formulations and, consequently, boost the development of new pharmaceuticals. Desirable properties include improvements in solubility and bioavailability of active compounds or improvement in the ability to cross biological barriers [46]. Nanoparticles can improve the stability and half-life of substances as well as facilitate the absorption of compounds with low solubility and hydrophobicity. Prolonged release of active agents, which reduces the frequency of drug administration and decreases toxic side-effects, and improved patient safety are also attributes of drug and peptide nanoencapsulation. Another positive aspect concerns drug administration by non-invasive routes that otherwise might be impractical for free substances, thus compromising patient treatment compliance [47]. All these features have stimulated the incorporation of drugs into nanomaterials to progress the development of effective drugs and biopharmaceuticals.

Studies have reported the utility of nanoparticles for a diverse number of clinical applications, such as improvements in the efficacy of anticancer therapies. This improvement is achieved by targeting therapeutic agents directly to the tumor [48]. Generally, chemotherapeutics specifically or indiscriminately target normal and tumorous cells, causing significant side-effects to the patient. In these cases, the strategy of directing therapy is highly attractive. By enhancing drug bioavailability and chemosensitivity, nanoparticle-mediated drug delivery may enhance the therapeutic efficacy against cancer cells and reduce damage to healthy cells and consequently attenuate side-effects, as seen with venom-loaded nanoparticles [49]. Other promising applications of nanocarriers include increased efficacy and safety of antimicrobial therapy. Nanocarriers and nanoparticles may also improve oral bioavailability and permeation of medicinal agents through the skin and the blood-brain barrier, as well as be useful as systemic transporters for gene therapy [50-55].

Specifically, regarding protein loading, nanoparticles may confer additional and beneficial properties to the use of these molecules as therapeutic agents. Nanostructures can confer protection against degradation in vivo, increase half-life, control release, and increase the selectivity and specificity of biological targets, thus improving the safety and efficacy of the therapy. This technology has enabled the delivery of proteins by alternative and more attractive routes, such as oral, nasal, pulmonary, and transdermal. Several polymeric, lipid, and inorganic nanocarriers have been developed for these purposes, and they are available for application [56]. Some formulations are already in the market or pharmaceutical development, such as a cyclosporine nanoemulsion (Neooral™) for oral prevention of organ rejection after transplantation and insulin liposomes for oral treatment of diabetes [57].

It is noteworthy that, despite nanotechnology-based methodologies demonstrating excellent potential for the formulation of therapeutic peptides and proteins, the clinical application of nanoparticles with this class of compounds is still in its early stages [56]. This scientific progress has led to the discovery of therapeutic proteins capable of effectively treating numerous diseases; however, the administration of peptide therapeutics is often hampered by several intrinsic limitations as mentioned above. These drawbacks have stimulated research in nanotechnology to promote the administration of peptides and proteins in more efficient modes. Furthermore, nanotechnology offers the possibility of incorporating peptides and proteins into nanocarriers that, among other advantages, protect cargo therapeutic polypeptides from the external environment and render them available in the respective sites of action [57, 58]. The use of nanostructured particles to deliver peptides, in addition to improving release kinetics, has the potential to minimize the effects of low selectivity, given that nanoparticles can be functionalized and can retain molecules in the nanostructure until the complete release at the desired site [59].
4.2. Nanocarriers and Nanoparticles

Several nanoscale systems have been used in research and development of new drug delivery platforms. Nanocarriers include inorganic nanoparticles, polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, liposomes, and dendrimers, among other materials [60]. These nanocarriers have attracted great attention because they present an exceptional surface area in relation to other systems, which confers excellent properties for the delivery of drugs such as high transport load capacity and controlled slow-release [61]. Nanocarriers or nanoparticles are small structures, between 1 and 100 nanometers in diameter, with the ability to transport other substances as a single unit [62]. Biologically and pharmacologically active substances can be loaded within these nanostructures, entrapped in the matrix or fixed to the surface and therefore can be formulated from the most diverse types of materials and methodologies, preferably those that are biocompatible with biological systems [63]. Polymeric nanoparticles are usually formed from synthetic polymers such as Poly(Lactide) (PLA), Poly(Lactide-Co-Glycolide) (PLGA), Poly(E-Caprolactone) (PCL), and poly(amino acids), or natural polymers, such as chitosan, alginate, gelatin, or albumin [64]. These nanoparticles can be distinguished into two main types: nanocapsules and nanospheres.

Nanocapsules are characterized by a vesicular structure that acts as a container for active substances to be carried, by which entrapped compounds remain in the aqueous or non-aqueous liquid core, surrounded by the solidified polymeric outer layer. Conversely, nanospheres appear as a spherical solid polymer mass in which the active substances are dispersed throughout the polymer matrix, both in the particle core and adsorbed to its surface [65]. Among the materials used to make these nanoparticles, PLGA is the most prominent in drug development. It is a biodegradable and biocompatible material approved by the FDA and the European Medicines Agency for drug delivery systems [66]. Its degradation by hydrolysis produces lactic acid and glycolic acid, is also biodegradable and its metabolites non-toxic, making this material an attractive resource for use in nanomedicine [11]. Chitosan nanoparticles have also received much attention for delivery of peptides, proteins, antigens, oligonucleotides, and genes for intravenous and oral administration. Similarly, chitosan is widely used in pharmaceutical research and industry as a vehicle for drug release and as a biomedical material. Chitosan is a bio-adhesive polysaccharide, is biodegradable, non-toxic, and compatible with biological tissues in numerous toxicity tests and research [67].

Physiologically tolerated, biocompatible, and biodegradable lipids also comprise nanocarriers that form nanostructures. If a nanostructured matrix is composed of solid lipids, at room temperature they form Solid Lipid Nanoparticles (SLN) [68], whereas if the mixture is composed of solid and liquid lipids they are called Nanostructured Lipid Carriers (NLC). NLCs represent a new generation of lipid-based carriers, with greater capacity to incorporate drugs in their structures [69]. Liposomes are another type of nanocarriers composed of lipids, more precisely phospholipids, forming one or more bilayers around an aqueous core [70]. In liposomes, organic drugs and polypeptides can be encapsulated within the aqueous core or the surface lipid bilayer. They have a structure similar to the cell membrane and are highly flexible, but their use is still limited by their low stability in the human body. Perfluorooctyl bromide nanoparticles form another type of nanostructure, consisting of a perfluorooctyl bromide (PFOB) core encapsulated within a phospholipid monolayer or a polymeric shell [71]. PFOB is a linear perfluorocarbon well known for its biocompatibility and good tolerability in humans and has been approved by the FDA. Such nanoparticles may be useful as delivery vehicles for therapeutic agents, which may be dissolved in the lipid or polymeric outer layer. Lipid-coated particles are preferred because they can facilitate the delivery of active substances through a lipid exchange or lipid mixture between the monolayer of the delivery system and the target cell membrane [71]. In addition, other materials such as cyclodextrins (cyclic oligosaccharides) [72], and inorganic nanoparticles, including gold, iron, silver, and silica nanoparticles, have been used to incorporate substances for therapeutic purposes [73, 74].

Nanostructured particles can be synthesized from different methods that are usually divided into two main categories, namely, bottom-up and top-down. The bottom-up methodology is a constructive approach, in which nanoparticles are formed from the union of smaller and simpler substances. In contrast, the top-down method is based on a destructive technique, in which larger substances are broken down into pieces and converted into nanoparticles [75]. An essential consideration to prepare nanoparticles is knowledge about the physicochemical characteristics of nanocarriers, such as size, surface characteristics, capacity of drug release, as well as their stability, given that these properties can directly influence bioavailability [76, 77]. By changing the synthesis method or by functionalizing the surface, for example, it is possible to design optimized nanocarriers to achieve specific purposes of drug administration [78], keeping in mind Paul Ehrlich’s principle of a drug acting on the right target. For instance, polymeric nanocapsules can be formed around a protein, generating a polymer coating, combining the robustness of inorganic nanoparticles with the flexibility of liposomes [79], while nanoconjugation (bio-nanoconjugation), the covalent linkage between the biomolecule and the nanocarrier, can create cell- or receptor-targeted nanoparticles [80]. Furthermore, nanoconjugation has the potential to reduce the toxicity of therapeutically active molecules, providing a mechanism to increase its effectiveness [12, 80]. Complete information regarding the types of nanocarriers, their structures, methods of preparation of nanoparticles, and indicated applications can be retrieved from specialized chapters in thematic publications [81-83].

4.3. Nanocarriers in the Cargo-Delivery of Venom-Derived Peptides

Advances in nanocarriers for the formulation of venom peptides include, especially, an improved ability to bypass membranes, better absorption by alternative routes such as oral and nasal, increased selective cytotoxicity of peptides (e.g., antitumor peptides), reduced toxicity toward normal cells, and increased half-life. In Table 2 [84-98], examples from the literature of venom-derived peptides formulated with
Table 2. Venom-derived peptide-loaded nanocarriers as biopharmaceuticals.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Biological Activity</th>
<th>Nanocarrier</th>
<th>Application</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exendin-4</td>
<td>GLP-1 agonist</td>
<td>Chitosan-coated PLGA nanoparticles</td>
<td>Increased transmembrane permeation <em>in vitro</em> and <em>in vivo</em>. Potential system for oral administration.</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipid-based nanoparticles</td>
<td>Increased transport and cellular uptake <em>in vitro</em>, and transport through the intestinal epithelium <em>in vivo</em>. Hypoglycemic effects in diabetic KKAY mice. A potential system for oral administration.</td>
<td>[85]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Solid lipid nanoparticles</td>
<td>Maintenance of the positive effects on insulin secretion without apparent cytotoxic effects.</td>
<td>[86]</td>
</tr>
<tr>
<td>Neurotoxin</td>
<td>Analgesic effect</td>
<td>PLA nanoparticles modified with chitosan</td>
<td>System for intranasal administration. Improved transport of the peptide to the brain through the blood-brain barrier.</td>
<td>[87]</td>
</tr>
<tr>
<td>Melittin</td>
<td>Cytolytic/antitumor activity</td>
<td>Perfluorocarbon nanoparticles</td>
<td>Potential for attenuating HIV infectivity, reducing toxicity against spermatozoa and vaginal epithelial cells <em>in vitro</em>.</td>
<td>[88, 89]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nano-liposomes with poloxamer 188</td>
<td>Preserves antitumor effect, reduced hemolysis <em>in vitro</em> and reduced signs of toxicity <em>in vivo</em>. Increased melittin half-life.</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zeolitic imidazolate framework-8 nanoparticles</td>
<td>Increased antitumor activity and reduced toxicity, <em>in vitro</em> and <em>in vivo</em>.</td>
<td>[92]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nanoassemblies of polymeric rigid core micelles</td>
<td>Maintenance of antitumor activity. Reduction of toxicity <em>in vivo</em>.</td>
<td>[91]</td>
</tr>
<tr>
<td>TsAP-1</td>
<td>Cytolytic/antitumor activity</td>
<td>Nanoassemblies of polymeric rigid core micelles</td>
<td>Increased antitumor activity.</td>
<td>[58]</td>
</tr>
<tr>
<td>Melittin/sodium dodecyl sulfate complex</td>
<td>Cytolytic/antitumoral activity</td>
<td>PLGA nanoparticles</td>
<td>Maintenance of antitumor effect <em>in vitro</em>.</td>
<td>[93]</td>
</tr>
<tr>
<td>NKCT1</td>
<td>Antitumor activity</td>
<td>Gold nanoparticles</td>
<td>Reduction of toxicity <em>in vitro</em> and <em>in vivo</em>. Potentiation of antitumor effects.</td>
<td>[12, 94]</td>
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<td></td>
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<td>PEGylated gold nanoparticles</td>
<td>Potentiation of antitumor effects.</td>
<td>[95]</td>
</tr>
<tr>
<td>ICD-85</td>
<td>Antitumor activity</td>
<td>Sodium alginate nanoparticles</td>
<td>Increased toxicity in tumor cells and decreased toxicity in normal cells <em>in vitro</em>.</td>
<td>[96, 97]</td>
</tr>
<tr>
<td>Bothrops toxin-II</td>
<td>Leishmanicidal activity</td>
<td>Liposomes</td>
<td>Selective toxicity against promastigotes and amastigotes of <em>Leishmania amazonensis</em>. Reduced toxicity to the host cell.</td>
<td>[98]</td>
</tr>
</tbody>
</table>

nanocarriers are listed with regard to the type of nanostructure and the improved properties that were achieved with this special, nanoscale formulation technology.

Exenatide (Byetta™) is a synthetic version of exendin-4. When launched in 2005 for the treatment of diabetes mellitus type 2, two injections per day were required [99]. Recently, a new version of exenatide encapsulated in PLGA microspheres (Bydureon™) was approved for clinical use; in this version, the gradual release of exenatide allows desirable therapeutic effects with injections administered weekly [100]. Despite this improvement, formulations for subcutaneous injections continue to be limited in their therapeutic applications, which is responsible for low adherence to the drug regimen, especially in the treatment of chronic diseases. In this sense, some studies have already reported delivery systems based on nanotechnology capable of improving the oral absorption of exendin-4.

Uncoated and chitosan-coated PLGA nanoparticles were designed to increase the transmembrane transport of exendin-4, reaching 8.9 and 16.5-fold increases, respectively, compared with non-encapsulated peptides, using a Madin-Darby canine kidney (MDCK) cell model [84]. The increased transport into the cells of this kind of functionalized nanoparticle is attributed to the net-positive charge of chitosan-coated PLGA nanoparticles that promote interaction with the negatively charged membrane of a target cell and facilitates the cellular uptake of the carrier peptide. Thus, this delivery strategy is useful not only to improve the oral ad-
ministration of exenatide but also of other drug cargoes. Similar results were found with exendin-4 encapsulated in lipid-based nanoparticles composed of an aqueous core with drug-loaded sodium cholate micelles and a mixed solid lipid shell [85]. The cellular uptake and transport of peptides were significantly increased in vitro. Exendin-4 was also readily transported through the epithelium, achieving a bioavailability of 12.7% in vivo, following intestinal administration in rats. The use of SLN may also be a promising alternative for the administration of exendin-4, as the encapsulation in these nanostructures was readily accomplished and maintained the effectiveness of the original peptide to stimulate glucose-dependent insulin secretion (incretin effect) in INS-1 cells in vitro, without inducing cytotoxic effects [86].

In relation to the oral administration of nanoparticles, size is one of the most important characteristics for absorption, distribution, and behavior in vivo. In vitro and in vivo cellular uptake of nanocarriers with a particle size around 100 nm is much more expressive than larger particles, such as microparticles. This observation can be explained by the mechanisms of nanoparticle translocation through the intestinal epithelium, which involves more efficient absorption in the Peyer’s patch [101].

Another example of peptide-loaded nanocarriers designed to overcome barriers has been ascribed to the encapsulation of an analogic peptide neurotoxin derived from the venom of the Asian cobra, Naja atra. Such analogic peptides can effectively block the acetylcholine receptor in the target membrane of host (or patient) cells, preventing neurotransmission, despite the low permeability of the peptide to blood-brain barrier translocation, limiting its bioactivity. Indeed, chitosan-modified PLA nanoparticles were formulated by the double emulsification solvent evaporation method for nasal administration, and studies in rats showed that neurotranspent in the brain was improved [87]. The physicochemical properties of chitosan allow enhanced transmucosal absorption, especially in the case of nasal delivery of polar drugs, including peptides and proteins. The use of nanocarriers has made possible the reduction of toxicity of many peptides toward non-target cells and tissues of biological systems. Concomitantly, nanotechnological formulations have also potentiated the therapeutic effect of compounds already in use.

A remarkable application of nanotechnology for toxicity reduction involves the encapsulation of melittin, a cytolytic peptide derived from the venom of the honey bee, Apis mellifera, that displays diverse biological effects in vivo, including anticoagulant effects. Melittin can insert into lipid bilayers to form pores that result in cell lysis. The load and transport of melittin into perfluorocarbon nanoparticles has been reported. PFBO-melittin was formulated by an oil-in-water emulsion, composed of a liquid perfluorooctyl bromide core and a phospholipid monolayer, forming a stabilizing interface with the aqueous medium. Perfluorocarbon nanoparticles were initially synthesized by sonication and microfluidization, and melittin was later incorporated into the outer lipid monolayer of this nanosystem. Melittin delivery was successfully achieved as confirmed by the ability to form pores in a dual-membrane model in vitro [102]. The encapsulation of melittin is justified by the non-selective cytotoxicity caused by this peptide against normal, non-targeted cells and erythrocytes, which produces hemolysis and precludes the use of melittin in therapy.

Using nanocarriers, the incorporation of melittin was reported to reduce its cytotoxicity against human spermatozoa and vaginal epithelial cells (VK2/E6E7) at concentrations shown to be adequate to restrain HIV infectivity [88-89]. Perfluorocarbon nanoparticles also reduced the hemolytic activity of melittin in vitro, and no apparent toxicity was demonstrated in mice after injection of a dose of nanoparticles equivalent to four times the LD₅₀ of free melittin. No significant changes in serum enzyme or electrolyte levels and no evidence of hemolysis or tissue damage in the liver, lung, kidney, and heart were observed. Nanoparticle-formulated melittin maintains toxicity against B16F10 melanoma cells in vitro, but at a much lower magnitude than free melittin, reflecting the slow release of the formulated nanosystem. In addition, nanoparticle incorporation reduced the clearance of melittin when administered intravenously to mice, resulting in a 10-fold increase in the amount of circulating melittin in the blood 2 hours after injection. Interestingly, the anticancer efficacy of melittin-loaded target-driven nanoparticles was similar to the non-targeted composites, indicating that such a loaded carrier may exert their anti-tumor effects by a nonspecific uptake mechanism by the tumor vasculature or by binding to overexpressed integrin receptor in tumor cells [90]. Other examples of melittin-nanocarriers include the inclusion in Poloxamer 188 nanoliposomes that also reduced toxicity in vivo, which produced less tissue damage and toxicity to liver cells, reduced neutrophil-mediated inflammatory responses, and reduced allergic responses in mice. At the same time, nano-liposomes maintained the therapeutic activity of melittin, as demonstrated by the induced apoptosis observed in liver carcinoma cells in vitro and in vivo, and the inhibition of hepatocellular carcinoma in the LM-3 xenograft tumor model [91]. Melittin-loaded zeolitic imidazolate framework-8 (ZIF-8) nanoparticles were also reported [92]. In another strategy, melittin was modified with an anionic agent, sodium dodecyl sulfate, by a hydrophobic ion-pairing technique and the complex was then incorporated into PLGA nanoparticles, which was active against MCF-7 breast cancer cells [93].

The formulation of melittin with different nanocarriers illustrates the versatility of this technology concerning the numerous possibilities of producing biopharmaceuticals with venom-derived peptides and nanoparticles. In addition, nanostructured melittin has been prepared in association with a second peptide. Incorporation of polymeric rigid core micelles into nano-assemblies enhanced the anticancer effects of melittin and the cytolytic peptide, TsAP-1, improving the therapeutic potential of the associated peptides in nanoparticles [58]. Another example of peptide association and preparation of a delivery nanosystem includes sodium alginate nanoparticles loaded with ICD-85. ICD-85 nanoparticles were developed by an ionic gelation method and a combination of three peptides with antiproliferative activities that are derived from the venom of the Iranian brown snake Agkistrodon halys and of the yellow scorpion Hemiscorpius lepturus [96]. The ICD-85 nanoparticle increased cytotoxicity against HeLa human cervical carcinoma cells in vitro compared with free ICD-85, with a significant inhibitory
effect on the growth of these cells. Moreover, in vitro assays showed that ICD-85 nanoparticles could significantly decrease the cytotoxicity toward healthy primary lamb kidney cells compared to the unencapsulated peptides, again demonstrating reduced off-target toxicity of peptide-loaded nanoparticles [97].

Liposome nanocarriers have been used to encapsulate venom peptides. Bothrops toxin-II (BthTX-II), an ASP49 phospholipase A2 derived from *B. jararacussu* venom, was formulated with lipid vesicles prepared by the extrusion method using dipalmitoylphosphatidylcholine, dipalmito-

<table>
<thead>
<tr>
<th></th>
<th>Biological Activity</th>
<th>Nanocarrier</th>
<th>Application</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Walterinnesia aegyptia</em> snake venom</td>
<td>Antitumor activity</td>
<td>Silica nanoparticles</td>
<td>Increased antitumor activity and reduced toxicity, in vitro and in vivo.</td>
<td>[49]</td>
</tr>
<tr>
<td>Russell's viper venom</td>
<td>Antigen</td>
<td>Chitosan triplyphosphate nanoparticles</td>
<td>Low toxicity. Potential adjuvant system for future medicines.</td>
<td>[103]</td>
</tr>
<tr>
<td><em>Naja naja oxiana</em> snake venom</td>
<td>Antigen</td>
<td>Chitosan triplyphosphate nanoparticles</td>
<td>Potential adjuvant system for future medicines.</td>
<td>[67]</td>
</tr>
</tbody>
</table>

4.4. Delivery of Crude Animal Venom Using Nanoparticles

In addition to isolated, pure venom-derived peptides, nanoparticles have also been used to encapsulate non-fractionated, crude venoms, as shown in Table 3. A combination of silica nanoparticles with the venom of the snake *Walterinnesia aegyptia* potentiated the growth arrest and apoptosis of human breast carcinoma cells (MDA-MB-231 and MCF-7) and prostate cancer cells (PC3 and LNCaP), in comparison with the non-formulated venom, without affecting normal cells. Similarly, these nanoparticles also reduced the volume of breast and prostate tumors in experimental animal tumor models. This reduction was twice as pronounced as that seen with the crude venom, without, however, causing significant side-effects on body weight - a predictive parameter of low toxicity in vivo [49]. In another nanosystem, chitosan triplyphosphate nanoparticles containing Russell's viper venom were formulated using the ionic gelation method. No significant morphological alterations were produced in normal cardiac cells treated with these nanoparticles. These venom-loaded nanoparticles displayed low cytotoxicity and caused less than 10% mortality of healthy cardiac cells [103]. The same type of nanoparticles was studied for encapsulation of the crude venom of the Indian cobra *Naja naja oxiana* to be used as an alternative to traditional adjuvant systems for future production of biological pharmaceuticals [67].

CONCLUSION

Venom-derived peptides have emerged as promising resources for the development of new biopharmaceuticals and diagnostic tools, particularly due to their ability to interact selectively and specifically with therapeutically relevant molecular targets. As with most peptide-based drugs, stability and bioavailability are critical conditions for achieving a high efficacy of peptides as therapeutics. Moreover, off-target effects may cause adverse reactions that are a matter of caution, particularly, in the case of venom peptides. The use of nanocarriers for drug delivery has shown to be advantageous for pharmaceutical formulation, allowing fine adjustments in the composition of nanocarriers to transport and release loaded compounds that are usually hydrophilic, structurally labile, and potentially toxic. The union of these two areas of knowledge, that is, toxinoology and pharmaceutical manufacturing, can boost the discovery of new therapeutic agents and advance how peptides are used therapeutically and diagnostically, translating this class of compounds, from the bench into final products with clinical applications. The theme of the present work enlightens the use of nanotechnology and nanocarriers to overcome some disadvantages of peptide administration, particularly venom-derived peptides with unique biological activities and pharmacological effects. Despite that, the majority of examples in the literature concern experimental studies, some formulations have already achieved clinical use, by which nanoparticles loaded or even functionalized with venom peptides have great potential.
to move forward in drug development platforms, as well as in the production of biopharmaceuticals for effective applications in pharmaceutical biotechnology.

CONSENT FOR PUBLICATION
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CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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