Advanced Drug Delivery Reviews 90 (2015) 101–118

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Toxins and derivatives in molecular pharmaceutics: Drug delivery and targeted therapy☆

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ARTICLE INFO

Available online 8 May 2015

Keywords:
Toxin
Protein
Peptide
Drug delivery
Targeted therapy
Glioma
Blood-brain barrier

ABSTRACT

Protein and peptide toxins offer an invaluable source for the development of actively targeted drug delivery systems. They avidly bind to a variety of cognate receptors, some of which are expressed or even up-regulated in diseased tissues and biological barriers. Protein and peptide toxins or their derivatives can act as ligands to facilitate tissue- or organ-specific accumulation of therapeutics. Some toxins have evolved from a relatively small number of structural frameworks that are particularly suitable for addressing the crucial issues of potency and stability, making them an instrumental source of leads and templates for targeted therapy. The focus of this review is on protein and peptide toxins for the development of targeted drug delivery systems and molecular therapies. We summarize disease- and biological barrier-related toxin receptors, as well as targeted drug delivery strategies inspired by those receptors. The design of new therapeutics based on protein and peptide toxins is also discussed.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Current and Forthcoming Approaches for Systemic Detoxification".

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http://dx.doi.org/10.1016/j.addr.2015.04.025
0169-409X/

1. Introduction

Toxins are a class of specific chemical substances that are capable of causing diseases on contact with or absorption by body tissues. Typically they interact with biological macromolecules, such as enzymes and cellular receptors, to exert poisonous effects. As a part of defensive and/or predation strategies, protein and peptide toxins have evolved in microbes, plants and animals for a very long time. Those toxins and their targets constitute a remarkable molecular world of complementarity and diversity. Over the past decades, our understanding of the mechanism of action of protein and peptide toxins has increased enormously. For example, a variety of fundamental studies contribute to the molecular definition and the discovery of important pathways in cell biology by unraveling interactions of toxins and their extracellular and/or intracellular targets. Those findings rapidly expand the biomedical applications of toxins, such as for tissue and organelle targeted drug delivery. Moreover, protein and peptide toxins are of interest in drug discovery because they can be directly used as therapeutic agents, serve as leads, and provide molecular scaffolds.

1.1. The diversity of protein and peptide toxins: cellular pathways, targets and frameworks

Bacteria and plants produce a variety of protein toxins. Among those, AB toxins have attracted much attention in the design of targeted drug delivery systems. AB toxins comprise the A and B subunits. The A subunit generally has enzymatic activity and exerts effects toward intracellular targets after entry into the cytosol. The B subunit contains more functional domains, binding to cell membrane receptors and facilitating intracellular transport of the A subunit. Regardless of target molecules, AB toxins have to enter the cytosol to exert their effects. They hijack all known endocytic routes to enter cells, which depend on the nature of receptors and may vary among cell types. Some protein toxins enter cells via clathrin-coated vesicles (such as diphtheria toxin and anthrax toxin), while others through the caveolae pathway (cholera toxin). Interestingly, the plant toxin ricin is internalized by all pathways including a putative clathrin- and caveolae-independent route, probably as a consequence of its binding to several receptors [1]. Protein toxins also behave quite differently after entry into endosomes (as described in Section 2.1).

Venomous animals from various phyla produce a large number of peptide toxins, which are often of small size and highly structured through a large density of disulfide bonds. They exert multiple functions toward a diversity of targets. Ion channels contain a variety of membrane proteins with a variable degree of functional and structural similarities and complexities, to which venomous protein toxins bind with different profiles of affinity and specificity. Highly structured mini-proteins (conotoxins and conopeptides) from the venom of cone snails constitute a big family of pharmacological probes. It is estimated that more than 100,000 conopeptides exist, while less than 0.1% of those mini-proteins have been identified and well characterized [2]. Of those characterized to date, a surprising number have been found to be highly selective for a diverse range of mammalian ion channels and receptors associated with pain signaling pathways, including the nicotine acetylcholine receptors (α-conotoxins), the noradrenaline transporter (χ-conopeptides), and sodium channels (μ-conotoxins), among many others [3]. Within the same voltage-gated ion channels, it is intriguing that toxins exert effects by precisely hitting specified sites. Sodium channels are molecular targets for several groups of neurotoxins. Binding to receptor at different sites (sites 1–6) strongly affects channel functions [4,5].

Over the past decades numerous studies have been conducted to decipher the functional and structural basis of toxin action, and our understanding of the mechanisms has increased enormously. Protein and peptide toxins that adopt a similar fold may exert unrelated functions, and vice versa. Ion channel blockers found in scorpion venoms consist of two major populations, including short-chain toxins (less than 40 amino acid residues) blocking potassium [6] and chloride channels [7], as well as long-chain toxins (60–70 amino acid residues) affecting sodium channels [8]. Although there is no sequence similarity among short-chain and long-chain scorpion toxins, they adopt a similar architecture comprising one α-helix and a three-stranded β-sheet (Fig. 1A). The cysteine-stabilized α-helix motif contains a Cys–X–X–X–Cys stretch of the α-helix bonded through two disulfide bridges with a Cys–X–Cys triplet of a β-strand. It also occurs in toxins purified from snake venoms. The functional derivation is precisely modulated by the middle loop of the "three fingered" toxins [9]. It suggests that a group of animals undergo a divergent evolution on conserved scaffolds, which may offer instrumental templates for drug design. On the contrary, toxins adopting unrelated architectures may act on the same targets. Both *kappa* conotoxin [10] (Fig. 1B) synthesized by *Conus purpurascens* and BgK toxin produced by sea anemone *Bunodosoma granulifera* [11] (Fig. 1C) are able to block potassium channels efficiently. However, their scaffolds are distinct from each other.

1.2. Targeted drug delivery and molecularly targeted therapy

The concept of "magic bullet" proposed by Paul Ehrlich in 1906 envisioned selective delivery of active agents, such as chemotherapeutics that are ubiquitously toxic to cancer cells and normal tissues, to the organs in question. It is ideal that those toxic chemicals are only delivered to tumor and/or tumor-related tissues to kill tumor cells with minimal side effects. Actively targeted drug delivery enables targeted and intracellular delivery of therapeutics to specified cells by active ligands. In addition, naturally occurring biological barriers always preclude the access of drug to target destinations. Targeted drug delivery may expedite traversal over such barriers. For example, gene drugs for intracellular targets or most of the drugs for central nervous system (CNS) diseases may need particularly targeting strategies to penetrate the lipid membrane of target cells and the blood–brain barrier (BBB), respectively.

Targeted therapy or molecularly targeted therapy, which is to molecularly manipulate the therapeutic targets that are critical to disease development and/or progression, acts as one of the major modalities of medical treatment of cancers. Molecularly targeted therapeutics block tumor growth by antagonizing specific targets (extracellular or intracellular) needed for carcinogenesis. Targeted therapy is expected to be more effective than conventional chemotherapy. For example, new drugs can interfere with signal-transduction pathways, downregulate proto-oncogenes involved in cancer-cell proliferation or block tumor angiogenesis. Imatinib, which targets the BCR–ABL oncogene [15], has demonstrated high efficacy and safety to treat advanced gastrointestinal stromal tumors [16] and chronic myeloid leukemia [17]. In some cases, molecularly targeted therapy and targeted drug delivery may be combined to enhance the targeting efficiency and/or to eliminate drug resistance. For instance, targeted drug delivery system may be used to improve intracellular transport of targeted therapeutics if necessary,