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Review

Toxins and drug discovery

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ABSTRACT

Components from venoms have stimulated many drug discovery projects, with some notable successes. These are briefly reviewed, from captopril to ziconotide. However, there have been many more disappointments on the road from toxin discovery to approval of a new medicine. Drug discovery and development is an inherently risky business, and the main causes of failure during development programmes are outlined in order to highlight steps that might be taken to increase the chances of success with toxin-based drug discovery. These include having a clear focus on unmet therapeutic needs, concentrating on targets that are well-validated in terms of their relevance to the disease in question, making use of phenotypic screening rather than molecular-based assays, and working with development partners with the resources required for the long and expensive development process.

1. Introduction

While venoms featured in several systems of traditional healing, the modern translation of toxins into medicines began in the 1940s with the introduction of tubocurarine into anaesthetic practice as a selectively acting muscle relaxant (Bowman, 2006). Tubocurarine is one of the key active ingredients in curare, the South American arrow poison. By binding to nicotinic acetylcholine receptors at the neuromuscular junction, tubocurarine blocks the transmission of excitatory signals from motor nerves to skeletal muscles, causing muscle paralysis. Use of tubocurarine allowed patients undergoing major surgery to be paralysed without using dangerously high doses of general anaesthetics. Although this revolutionised anaesthetic practice, the search soon began for new agents that lacked the cardiovascular side effects of tubocurarine. Since tubocurarine was known to have a relatively rigid core structure carrying two functional groups, most discovery work focused on synthetic compounds with curarimimetic actions: the toxin provided the template for drug design. Relatively little work involved explorations of other toxins that could cause paralysis. However, the most successful of the new muscle relaxants, atracurium, did draw on naturally-occurring curare-like alkaloids (Stenlake et al., 1983). Two relatively innocuous moieties were chemically linked to form the active molecule. The chemical bridge was designed to break down rapidly in plasma in order to provide elimination that was not dependent on liver or kidney function and to give a short-acting agent to facilitate the control of the duration of the paralysis. By chance, atracurium lacks the cardiovascular side effects of other muscle relaxants (blockade of nicotinic receptors in sympathetic ganglia that leads to a pronounced fall in blood pressure, and/or block of muscarinic cholinoceptors innervated by the cardiac vagus that could trigger arrhythmias). Atracurium was introduced in 1983, followed by cis-atracurium (a defined isomer) in 1995.

Other sources of arrow poisons, notably extracts of frog skin, were studied in the 1970s and '80s. While many compounds with interesting pharmacological actions were discovered (Daly, 1982; Philippe and Angenot, 2005), none has led to a successful medicine. However, the discovery of epibatidine and its analgesic effects indicated that neuronal nicotinic receptors could be a possible therapeutic target. Structurally related compounds were tested by the Abbott company, including tebanicline (ABT-594) that reached phase II clinical trials before being dropped because of its side effects (Arneric et al., 2007).

There was, however, a notable success from research on snake venoms, namely the development of captopril, the inhibitor of angiotensin converting enzyme (ACE). This work was based on small peptides from the venom of the South American snake *Bothrops jararaca* that were known to potentiate the action of bradykinin (for reviews, see Opie and Kowolik, 1995; Camargo et al., 2012). Although bradykinin potentiating peptides are not toxins in the sense of having a potentially lethal action, they do come from a venom of a snake that is dangerous to humans. Work in Brazil and London explored the concept that bradykinin potentiating peptides

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could inhibit the enzyme that was responsible for the production of vasoactive angiotensin (Smith and Vane, 2003). The hypothesis was that systemic blood pressure would be lowered by blocking angiotensin converting enzyme. This was demonstrated experimentally and in humans with the synthetic bradykinin potentiating peptide teprotide in 1971. However, teprotide had to be injected, an obvious disadvantage for treating patients with high blood pressure. Considerable effort led to the orally acting ACE inhibitor captopril, which was introduced in 1981 (Cushman et al., 1977). Since then, many follow-up compounds have been introduced.

2. More recent successes

Captopril's success is generally credited as triggering the recognition that venoms could be the source of new medicines (Harvey, 1992; Lewis and Garcia, 2003; Fox and Serrano, 2007; Shaw, 2009; King, 2011; Koh and Kini, 2012; Takacs and Nathan, 2014), but further successes have been rare. While snake venoms were recognised as the source of enzymes with specific actions on many of the components in the blood clotting cascade (Kornalík, 1991), there were no further developments of non-enzymatic compounds until the almost simultaneous introduction of eptifibatide and tirofiban in 1998. These act on GPIIb/IIIa integrin receptors on blood platelets to prevent platelet aggregation and thrombus formation. Clinically, they are used in patients with acute coronary syndrome and in high-risk patients undergoing coronary interventions. Both compounds owe their existence to research on snake venoms. Eptifibatide is a synthetic cyclic heptapeptide that mimics the action of a much larger peptide (73 amino acids) found in the venom of the southeastern pigmy rattlesnake *Sistrurus miliarius barbouri*. Tirofiban is not a peptide but it is based on a 49-residue polypeptide from a snake venom, echistatin from the saw-scaled viper *Echis carinatus*. Both compounds were designed to mimic the RGD sequence that is the recognition motif for binding to GPIIb/IIIa integrin receptors (Hashemzadeh et al., 2008).

More recently, venoms from sources other than snakes have attracted attention. Venoms from marine cone snails (*Conus*) have been proposed as promising sources of new drug leads (e.g., Lewis and Garcia, 2003; Twede et al., 2009; Essack et al., 2012; Vetter and Lewis, 2012) because they contain a rich variety of small peptides with diverse pharmacological actions. Ziconotide, the synthetic version of the venom peptide MVIIA from *Conus magus*, was approved by the FDA in 2004 for treating patients with intractable pain. The compound selectively blocks N-type calcium ion channels ($Ca_v2.2$); when administered intrathecally, it can reduce pain transmission in the spinal cord (Pope and Deer, 2013).

Sometimes included as successes in drug discovery and development from venom components are compounds inspired by molecules that are not exactly toxins and from sources that are not exactly venoms. Examples include variants of hirudin, the anticoagulant thrombin antagonist from the saliva of the medicinal leech, *Hirudo medicinalis* and exenatide, the GLP-1 agonist peptide from the saliva of the Gila monster lizard, *Heloderma suspectum* that is in use as an anti-diabetic agent (King, 2011; Takacs and Nathan, 2014).

Arguably, the biggest successes in translating toxins to products in recent years have been the developments from the microbial botulinum toxins. Both botulinum toxins A and B have been approved for clinical use to treat patients with a variety of conditions caused by over-activity of neurones. By restricting the toxins' actions by localised injections and through their highly selective uptake into particular nerves, botulinum toxins have been used successfully in, e.g., strabismus, blepharospasm, dystonias, hyperhidrosis and migraine (Abrams and Hallett, 2013; Matak and Lacković, 2014). Of course, many sales are derived from the use of botulinum preparations for cosmetic purposes, but further therapeutic applications have been proposed (Dolly et al., 2011). In recent developments, a topical formulation of botulinum toxin A (RT001) was efficacious in a double-blind trial to treat facial wrinkles (Glogau et al., 2012) and another topical formulation (ANT-1207) is currently in phase II clinical trials (http://clinicaltrials.gov/show/NCT01293552; http://clinicaltrials.gov/show/NCT01358695).

3. Trials and tribulations

Earlier reviews on the potential of toxin-related compounds have lists of products in clinical trials (King, 2011; Takacs and Nathan, 2014). Unfortunately, several of these products have since been dropped, illustrating how difficult it can be to go from promising effects in animal studies to beneficial and side-effect-free actions in humans.

Following the example of the conopeptide ziconotide, other *Conus* peptides have been pursued. A calcium ion channel blocker from *Conus catus*, ω-conotoxin CVID (variously named AM336, CNSB004 and leconotide) failed in clinical trials because of side effects encountered; these trials involved intrathecal administration, but the peptide was to be tried again using intravenous administration (Kolosov et al., 2010). However, the company responsible for the development, Relevare Pharmaceuticals, is in liquidation (https://insolvencynotices.asic.gov.au/browsesearch-notices/notice-details/Relevare-Pharmaceuticals-Ltd-142658259) The α-conotoxin Vc1.1 (ACV1) from *Conus victoriae* failed because of lack of efficacy: while this compound was identified as an antagonist of subtypes of neuronal nicotinic cholinoceptors, it was subsequently shown to have analgesic potential by acting through a rather different mechanism, that of activating GABA-B receptors and causing a decrease in calcium ion currents in nociceptive neurones (Adams and Berecki, 2013). Xen2174, the synthetic analogue of the χ-conopeptide Mr1A from *Conus marmoreus*, inhibits noradrenaline reuptake in neurones and has profound analgesic activity in animal studies (Lewis, 2012); however, it was also found to have dose-limiting toxicity in humans despite early promise in Phase I trials (Groeneveld, 2013).

Chlorotoxin from the venom of the scorpion *Leiurus quinquestriatus* was first identified as a blocker of some chloride ion channels and then found to inhibit matrix metalloproteinases (Deshane et al., 2003). It showed promise as a means to identify glioma tumour cells and, potentially, as a way to localise anti-cancer agents to such tumour cells (Wu et al., 2010). However, clinical trials with chlorotoxin and conjugates with ^{131}I appear to have been suspended (http://clinicaltrials.gov/ct2/show/NCT00733798) following a takeover of the development company.

Some compounds featured in earlier review articles do still appear to be under active investigation. A snake venom natriuretic peptide DNP (from green mamba *Dendroaspis angusticeps*) has been fused with human C-type natriuretic peptide to provide a molecule, CD-NP or cenderitide, that activates both A and B forms of the natriuretic peptide receptor, with the expectation that this would give both improved efficacy and a longer duration of action in patients with heart failure (McKie et al., 2010; Vink et al., 2012). A clinical trial assessing the pharmacokinetics, pharmacodynamics, safety and tolerability of subcutaneous administration of cenderitide in patients with chronic heart failure has been completed and a pilot study for the preservation of left ventricular function in patients after myocardial infarction is underway (https://clinicaltrials.gov/ct2/show/NCT02071602).

A synthetic peptide ShK-186 related to the potassium ion channel blocker ShK from the sea anemone *Stichodactyla helianthus* (Chi et al., 2012) is also in early-stage clinical trials (company website: http://www.kinetabio.com/autoimmune.html). The