In search of venomous cures

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From snakes to sea anemones, the rich diversity of poisonous creatures is slowly yielding compounds that act as useful leads to new therapies

Poisonous creatures may not seem the obvious place to look for medicine, but components of venoms from many different animals have found therapeutic uses. This is not restricted to historical approaches involving 'eye of newt and toe of frog' types of mixtures of exotic natural products. It includes the development of important therapeutic agents such as the ACE inhibitors (angiotensin-converting enzyme) used to treat high blood pressure. The prototype ACE inhibitor captopril was developed from the lead provided by small peptides isolated from the venom of the viper snake *Bothrops jaracusa*.

Venoms are complex mixtures of (usually) peptides and proteins. Their effects can be complicated because the components can have different actions and because some of the components may act in synergy. However, the individual constituents (the toxins) often have highly specific and very potent effects at targets within the body.

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Shellshocked: the venom from Conus *snails has provided leads to analgesics*

Toxins can be useful experimental tools for studying physiological processes. This is because they are better than available synthetic compounds at discriminating between different genetically related subtypes of ion channels or receptors on cells. For example, the neurotransmitter acetylcholine is used by many different nerves to control diverse physiological functions throughout the body. It acts on two major classes of recognition sites — the nicotinic and muscarinic receptors — that were originally distinguished by their differential sensitivity to the natural compounds nicotine and muscarine. However, molecular biology has revealed five genetically distinct forms of muscarinic cholinoceptor that are not easily distinguished by known drugs. Fortunately, there are muscarinic toxins from mamba snake venoms that bind exclusively to the m1 subtype of muscarinic cholinoceptor while having negligible effects on the closely related m2, m3, m4 or m5 subtypes.[1] Similarly, the highly complicated family of voltage-activated K^+ channels that control the excitability of nerve cells may be teased apart in functional experiments using small protein toxins from snake, scorpion and sea anemone venoms.[2] Additionally, peptide toxins are usually highly structured and very stable molecules. Their three-dimensional structures can be solved by X-ray crystallography or NMR spectroscopy, and that structural information can be used in the design of smaller analogues.

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Analgesic peptides and their analogues

The ω-conotoxins from venoms of various species of *Conus* marine snails block voltage-activated Ca^{2+} channels in excitable cells, such as nerves and muscles. The known toxins are highly homologous peptides containing 25–29 amino acid residues and three disulphide bonds. However, different toxins have different selectivities for the different subtypes of Ca^{2+} channel that control the release of neurotransmitter or modulate the contractions of different types of muscles.[3] ω-Conotoxin MVIIA is specific for N-type channels, which are particularly important in the pain fibres within the spinal cord. Synthetic ω-conotoxin MVIIA, or ziconotide, is in advanced clinical trials for treating severe pain. When administered into the spine, ziconotide acts on N-type Ca^{2+} channels in nerve cells in the spinal cord responsible for transmitting pain stimuli. Blocking these channels reduces the amount of neurotransmitter released from the nerve endings and therefore reduces the strength of the signal.

The results of full trials are awaited, but preliminary findings are encouraging. For example, in one published case study, ziconotide gave complete pain relief to a patient who had suffered from intractable 'phantom limb' pain after an amputation 23 years previously. Side effects were minimal and were decreased by adjusting the dose of ziconotide.[4]

Naturally occurring ω-conotoxins and hybrid structures have been compared for effects on N-type and P/Q-type Ca^{2+} channels. Detailed NMR analysis gave insights into the structure of the pharmacophore responsible for selective binding to N-type channels,[5] leading to the possibility of designing small molecule mimics. However, the knowledge of the effectiveness of ziconotide on various animal models inspired Michael Rafferty and colleagues

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The sawscaled viper: disintegrins from snake venom can prevent blood clotting

at Parke-Davis to screen for low-molecular-weight compounds with selective actions on N-type Ca^{2+} channels.

This approach is beginning to be successful, and one compound from a series of 4-benzyloxyanilines was particularly effective in a variety of functional models for pain and epilepsy.[6] Unlike ziconotide, this compound blocks nerve impulses in sensory neurones by blocking Na^+ channels at similar concentrations to those that blocked N-type Ca^{2+} channels. If the apparently greater efficacy of this compound as an analgesic holds up in further *in vivo* testing, this may prompt searches for compounds with a mixed blocking effect.

From snake venoms to superaspirin

Many snake venoms disturb blood coagulation and a great many active components have been isolated and studied for their actions on different parts of the coagulation cascade. For a number of years, attention has focused on the so-called disintegrins that bind to a surface receptor on blood platelets (glycoprotein GPIIb/IIIa, or integrin $\alpha_{IIb}\beta_3$) and prevent platelets binding to fibrinogen, which is the basic step in blood clotting. In other words, disintegrins block platelet aggregation. Workers at COR Therapeutics in South San Francisco and Merck & Co (New Jersey) concentrated on two particular disintegrins, barbourin (from the South Eastern pygmy rattlesnake *Sistrurus miliarius barbouri*) and echistatin (from the sawscaled viper, *Echis carinatus*), respectively.

Most of the disintegrins contain the amino acid sequence RGD (arginine–glycine–aspartic acid), which is thought to be at the centre of the interaction between fibrinogen and platelet GP IIb/IIIa. Barbourin is unusual in having lysine instead of arginine in this sequence, and this may lead to different specificity against different integrins. Scarborough and colleagues at COR Therapeutics examined many small cyclic analogues of barbourin and discovered eptifibatide (Integrilin), which is a cyclic hexapeptide inhibitor of GPIIb/IIIa binding to fibrinogen.[7]

Workers at Merck & Co also made cyclic peptides as mimics of native disintegrins, but they managed to develop nonpeptide analogues. They found that active compounds required a positive charge at one end of the molecule (they used an amino group), a hydrophobic group in the middle, and a negative charge (from a carboxylate group) at the other end. The resultant compound was tirofiban (so-called because of the use of tyrosine as the scaffold for the functional groups).

Eptifibatide and tirofiban belong to a new class of drug — the 'fibans' — which are under extensive clinical trials in patients (more than 18,000 enrolled) thought to be at risk of a heart attack. When used alone or in combination with other agents, the fibans reduce the incidence of death or serious coronary events.[8]

Other applications of disintegrins

Although much of the interest in the fibans stems from their specificity for GP IIb/IIIa, there are other snake venom proteins with inhibitory actions at other integrins, opening up additional therapeutic targets. For example, a group in Taiwan has reported that accutin from the hundred-pace snake (*Agkistrodon acutus*) — presumably different from the previously characterised thrombin-like protease called 'acutin' from the same venom — blocks integrin $\alpha V\beta 3$ on endothelial cells lining blood vessels

Tony Stone Images

Spitting distance: the saliva from Gila monsters could lead to treatments for type II diabetes and obesity

and prevents angiogenesis (blood vessel formation) *in vitro* and *in vivo*.[9] Such a mechanism of action could provide a useful cancer agent by preventing tumours from establishing their blood supply by growing new vessels.

A monstrous way to lose weight?

The salivary secretions of the Gila monster (*Heloderma suspectum*) have small proteins (extendins) with structural similarities to glucagon-like peptide.[1] They bind to the GLP-1 receptor and stimulate the release of insulin from pancreatic beta cells. Unlike glucagon, their hypoglycaemic effects can last several hours. Extendin-4 is being studied extensively in animal models of type II diabetes (non-insulin dependent) and obesity. Very low doses (<0.1μg/kg) of extendin-4 have prolonged actions in diabetic rats and mice, both acutely and chronically, leading to improved control of blood sugar levels and to weight loss.[10] Extendin-4 has also been demonstrated to be effective in diabetic Rhesus monkeys, suggesting that it should also work in humans.

Newer agents are undoubtedly waiting to be discovered

More to come?

Existing agents may find additional applications, and newer agents are undoubtedly waiting to be discovered. In terms of new applications, the thrombic protease ancrod from the venom of the Malayan pit viper (*Calloselasma rhodostoma*) has already been used to treat acute ischaemic stroke because of its ability to convert fibrinogen into soluble fibrin products and to stimulate the release of tissue plasminogen activator from blood vessel walls. More recently, ancrod has been tested in the treatment of patients after heart attack.

On a less medical note, botulinum toxin not only finds a use in the treatment of unwanted muscle spasms in conditions such as blepharospasm, but it is also being used in cosmetic surgery to remove facial wrinkles by relaxing superficial muscle groups. If you are worried about those crow's-feet and would like to find out more about the usefulness of botulinum toxin, try the Internet (for example, www.webplastics.com/botoxcoll.htm).

In terms of new agents, it is important to remember that most venoms have not yet been studied scientifically, let alone been used in a drug discovery programme. All spiders are venomous, but only a few of the hundreds of species have been examined. Similarly, with the cone snails: only a handful of species out of the several hundred known species have been subjected to detailed study. Sooner or later, animal venoms can be expected to provide new and surprising therapeutic agents.

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